EFFECTS OF ADULT SALMON CARCASSES ON THE ENERGY ALLOCATION STRATEGIES OF JUVENILE SALMONIDS

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Abstract

When adult salmon return to their natal streams to spawn they deliver energy in the form of carcass tissues and eggs. Currently, the effect of this marine-derived energy on the growth and energy allocation strategies of juvenile salmonids is unknown. This thesis examined the effects of marine-derived energy on the growth and energy allocation strategies of juvenile coho salmon and resident Dolly Varden. Fatty acid analysis was developed as a tool for monitoring the flow of marine-derived lipids and hence energy from carcass tissues to consumers in laboratory and field settings. Fish in these settings were examined before and after the arrival of adult salmon carcasses in their respective habitats. The allocation of protein and lipid was monitored in concert with the fatty acid analysis. In addition, the effect of different diets on fasting of wild coho salmon was studied to determine how marine-derived diets might influence over winter survival. Marine-derived energy was acquired by juvenile salmonids through both direct and indirect processes. Direct acquisition entailed consumption of marine-derived lipids or short trophic linkages between carcass tissues and consumers. Indirect acquisition was typified by long trophic linkages between consumers and carcass tissues in which marine lipids were incorporated by consumers after marine-derived lipids permeated food webs. The benefits of consuming marine-derived lipids depended on the method of acquisition. Fish that directly acquired marine-derived lipids altered their energy allocation strategies by storing greater amounts of lipid; allowing them to maintain elevated metabolic rates over winter and start spring in a high nutritional state. In contrast, indirect acquisition of marine-derived lipids afforded fish few benefits. These fish survive winter by down

regulating metabolic rates and start spring in a low nutritional state. The ubiquity of direct acquisition by coho salmon and variable routes of acquisition in Dolly Varden suggest that the presence of carcass tissues may serve to reinforce anadromy among juvenile salmonids rearing in streams.

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"Do not tell fish stories where the people know you; but particularly, don't tell them where they know the fish"

Mark Twain

If you have ever been to southeast Alaska you will immediately recognize the truth in Twain's observation. In southeast Alaska any conversation among friends invariably turns to fish. It has been a long time since I arrived here; a time when I could not tell a salmon from a stickleback. So it is with particular pride that I submit this fish story to a community that knows the fish. A project of this magnitude could never be completed alone, yet somehow I get the honor of being its sole author. That honor is distinctly unfair to my colleagues at NOAA's Auke Bay Laboratories, the University of Alaska and the Kachemak Bay Estuarine Research Reserve. A large number of people have contributed to this work. Some made contributions by helping with the sampling and processing, others offered constructive criticism and a few just made my life more comfortable. Perhaps most surprising is the number of new personal relationships I developed as a direct result of this work. I especially want to thank my best friend, Bonita Nelson. I am anxious to see where our lifelong conversation takes us next. I also wish to thank: Steve Baird, Robert Bradshaw, Matt Dietrick, Wyatt Fournier, Dugan Greenwell, Larry Holland, John Hudson, John Kennish, Marie Larsen, Jacek Maselko, Jeep Rice, Dan Rinella, Lawrence Schaufler, Fletcher Sewall, Mike Stekoll, Sherry Tamone, JJ Vollenweider, Coowe Walker and Mark Wipfli. I could not have done it without you.

Chapter I

General Introduction

Purpose of this thesis

My primary objectives for this thesis are to develop tools for understanding energy flow through food webs and the life history implications of different energy allocation strategies. These tools are needed by fishery managers if they are to develop ecosystem approaches to fisheries management. In this thesis I develop these tools by examining how juvenile salmonids in fluvial habitats use the energy delivered by adult salmon. The interaction between adult salmon and juvenile salmonids is an especially pertinent model for integrating trophic interactions with energy allocation strategies. Adult salmon acquire the bulk of their mass in marine ecosystems. When they return to their natal streams to spawn, they die and their mass is catabolized by producers and consumers in riparian ecosystems. At the same time, energy allocations made by juvenile salmonids directly influence their ability to survive winter and smolt. Two features of the interaction between adult salmon and juvenile salmonids make the interaction an excellent model for tracing trophic interactions. First, the ecology and life history of juvenile salmonids are reasonably well understood and alterations in the energy allocation strategies of juvenile salmon can be placed into a well defined context. Second, there are significant differences in the fatty acid compositions of carbon-reducing autotrophs in riparian and marine ecosystems. These differences have the potential to facilitate identification of the ultimate sources of energy consumed by juvenile salmonids

and thereby offer the opportunity to monitor energy flow. Each of these features is described in more detail below.

Tools for understanding trophic interactions

It is necessary to distinguish between the various sources of energy entering freshwater habitats in order to determine the value of marine-derived energy to juvenile salmonids. Freshwater algae and terrestrial plants are carbon reducing autotrophs are the initial source of energy at the base of freshwater food webs. Carbon reduced by freshwater algae is referred to as an autochthonous energy source, because reduction relies on nutrients available in the water body. Reduced carbon that derives from terrestrial plants is considered to arise from allochthonous sources. This latter carbon arrives directly in the form of vegetable matter that falls into streams or indirectly in the form of terrestrial consumers entering the stream. For the purposes of this thesis all of these energy sources are chemically indistinguishable and are referred to as terrestrial sources. In contrast, some streams receive marine nutrients, delivered when anadromous fishes return to spawn. In this case, a significant amount of carbon is introduced that has been reduced by marine phytoplankton. This carbon source is also considered an allochthonous source. For the purposes of this thesis it is referred to as a marine source. The importance of marine carbon to riparian habitats is currently an active field of research (Gende et al. 2002; Naiman et al. 2002; Schindler et al. 2003).

Methods for identifying the energy sources supplying consumers have historically been limited to stomach content and stable isotope analyses. Stomach contents can contain only the most recently consumed prey and provide no information

about the initial source of the carbon. Stomach content analysis is best used for identifying the proximate sources of energy. Researchers seeking to identify the initial sources of energy used by consumers have recently begun relying on isotopic analysis. This approach relies on differential fractionation of naturally occurring carbon isotopes by carbon-reducing autotrophs. Marine organisms are typically enriched in ^{13}C relative to organisms in freshwater, so juvenile fish found to be consuming marine-derived carbon have more ^{13}C than fish relying on terrestrial sources of carbon (Hicks et al. 2005).

Fatty acid analysis is a third method that can be used to discriminate between energy sources. Ingested fatty acids are stored with little or no modification in lipid reserves (Budge et al. 2006) unless they are immediately catabolized. In addition, fatty acid compositions are species specific (Iverson et al. 2004). The fatty acid composition of a consumer therefore integrates all of the fatty acids acquired from its prey in proportion to the lipid found in each prey item. Consequently, identification of the source of fatty acids in a consumer accomplishes with identification of the source of its energy reserves. The application of these observations to qualitatively identify energy sources has been generally accepted for several decades (Budge et al. 2006). The use of fatty acid analysis is most powerful when potential energy sources have distinct fatty acid compositions. For example, the fatty acid compositions of marine and freshwater fishes are distinct due to differences in the production of polyunsaturated fatty acids by terrestrial plants, freshwater and marine algae (Napolitano 1999). Consequently, the fatty acid compositions of salmon depend on whether they are found in freshwater or saltwater

(Henderson and Tocher 1987). These differences were the basis for inferring the energy sources consumed by stream fauna (Heintz et al. 2004; Volk 2004).

Potential effects of adult salmon on juvenile salmonids

What is known about the effects of returning adult salmon on the energetics of juvenile salmonids stems from experiments involving carcass additions to natural or artificial streams. The earliest of these manipulations indicated that the presence of carcasses increased the density of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead (*O. mykiss*) in Washington streams (Bilby et al. 1998). In a later study, coho salmon in artificial streams (mesocosms) had higher growth rates when carcass tissues were present. This growth effect was asymptotic with carcass density, suggesting an optimal density of carcasses for growth (Wipfli et al. 2003). The presence of carcasses in these artificial streams also led to increased levels of lipid and triacylglycerols in coho salmon (Heintz et al. 2004). Increased growth and lipid content was also observed among Dolly Varden char (*Salvelinus malma*) and cutthroat trout (*Salmo clarki*) after adding carcass tissues to a non-anadromous stream (Wipfli et al. 2003). The results of these manipulations are consistent with observations made on coho salmon rearing in beaver ponds that naturally received carcasses. Those coho salmon also had increased growth relative to those in ponds that did not receive carcasses (Lang et al. 2006). Concerns over health issues associated with distributing decaying fish tissues in streams led to evaluations of the effects of pathogen-free carcass products on growth of coho salmon and cutthroat trout. These studies confirmed that addition of these carcass products led to increased growth and lipid levels in salmonids (Wipfli et al. 2004). More recently,

carcass additions to fenced-off stream sections resulted in both increased size of coho salmon over winter and larger smolt sizes (Giannico and Hinch 2007).

How are these effects accrued?

The increased growth and lipid reserves observed in juvenile salmonids probably results from consumption of carcass tissues or eggs. In the earliest of the previously described studies, juvenile salmonids captured near carcasses were found to be consuming salmon eggs (Bilby et al. 1998). Similarly, in some British Columbian streams carcass tissues are the primary source of particulate organic carbon (Johnston et al. 2004). Following the arrival of adult sockeye salmon, juvenile salmonids in southwestern Alaska shifted their diets from aquatic invertebrates to salmon eggs (Eastman 1996). These observations of direct consumption of carcass tissues are corroborated by isotopic studies, which document the uptake of marine-derived carbon in juvenile salmonids (Bilby et al. 1998; Chaloner et al. 2002; Gregory-Eaves et al. 2007; Hicks et al. 2005; Kline et al. 1990).

In addition to directly providing food to juvenile salmonids, spawning salmon can affect the availability of invertebrate prey in streams by dislodging significant numbers of benthic invertebrates during redd excavation (Lessard and Merritt 2006; Minakawa and Gara 2003; Moore and Schindler 2008; Peterson and Foote 2000). Excavation results in increased drift, placing invertebrates at increased risk of predation (Peterson and Foote 2000). Arctic grayling (*Thymallus arcticus*) focused their consumption on insects dislodged by spawning sockeye salmon rather than consuming carcass tissue (Scheurell et al. 2007). However, these effects are likely to be limited to the relatively limited time

period in which adult salmon are actively spawning. After spawning, insect density and biomass is often diminished (Chaloner et al. 2004; Lessard and Merritt 2006; Minakawa and Gara 2003; Moore and Schindler 2008).

The nutritional value of carcass tissue to invertebrates may benefit juvenile salmonids indirectly by improving prey quality. Benthic invertebrates, particularly chironomids, colonized naturally occurring carcasses and those placed in streams in southeastern Alaska (Chaloner and Wipfli 2002). Other benthic vertebrates will consume carcass tissues as indicated by isotopic studies (Bilby et al. 1998; Chaloner et al. 2002; Hicks et al. 2005; Kline et al. 1990). Consumption of carcass tissue may support enhanced growth and therefore invertebrate biomass (Minakawa et al. 2002; Zhang et al. 2003). The presence of carcasses in artificial streams resulted in increased abundance, biomass (Chaloner and Wipfli 2002) and density (individuals/m^2) of benthic invertebrates (Wipfli et al. 1998). In the latter study, a similar effect of carcasses on invertebrate density was also observed in carcass-enriched sections of a nearby stream. In addition, the presence carcasses may increase the availability of alternative prey. Terrestrial dipterans colonized the dry portions of carcasses in southwestern Alaska and ultimately became an important prey item for juvenile salmonids (Eastman 1996; Scheurell et al. 2007).

Indirect effects of fish carcasses on food supply may also be important to juvenile salmonids, but these effects are not well documented. For example, the massive amount of food made available by spawning and dying salmon may result in less competition between fish species. In southwestern Alaska, Arctic grayling and rainbow trout

maintained similar diets until adult sockeye salmon arrived, after which the grayling increased their consumption of benthic invertebrates and rainbow trout shifted their diets to salmon carcass tissues (Scheurell et al. 2007). This competitive release may be inferred from observations of increases in the density of juvenile salmonids coinciding with the return of adult salmon (Bilby et al. 1998; Eastman 1996). Increases in fish density are only feasible if there is a food surplus. Simultaneously, when juvenile salmonids recruit to areas where carcasses are available, they leave behind other individuals that may enjoy increased access to food. Tagged juvenile salmon that moved from one location to another area where adults were spawning were generally the larger individuals in the tagged populations (Eastman 1996). Presumably, smaller individuals in locations vacated by older/larger fish experienced decreased competition for food and decreased predation risk.

Other indirect effects on juvenile salmonids may accrue if carcasses cause increased production in receiving streams. Adult salmon deliver important nutrients, such as phosphorous and nitrogen, to streams and increase biofilm biomass and chlorophyll-*a* (Chaloner et al. 2002; Johnston et al. 2004; Minakawa and Gara 1999; Wipfli et al. 1998). The availability of these nutrients often controls productivity in stream ecosystems (Ashley and Slaney 1997). The abundance of sockeye salmon determined primary production in streams in British Columbia (Johnston et al. 2004). These marine derived nutrients are thought to fuel a positive feedback mechanism by which increased numbers of fish carcasses lead to increased juvenile production. This principle was documented in sediment cores taken from Alaskan lakes (Gregory-Eaves et al. 2003). Harvest of adult

salmon and other anthropogenic impacts on salmon survival are thought to have disrupted this feedback mechanism in the northwestern United States resulting in lost productivity (Gresh et al. 2000).

Potential benefits of increased growth for juvenile salmonids

An immediate benefit of rapid growth in juvenile salmonids is a disproportionate increase in the amount of lipid. Increased growth is consistent with large increases in the lipid reserves in Atlantic silversides (*Menidia menidia*) (Schultz and Conover 1997) rainbow trout (Post and Parkinson 2001) and striped bass (*Morone saxitilis*) (Hurst and Conover 2003) prior to winter. In each of these cases, the lipid levels increased hyperallometrically in relation to fish size. The magnitude of this hyperallometry depends on fish size (Post and Parkinson 2001), season (Hurst and Conover 2003) and habitat (Schultz and Conover 1997). The effect of these dependencies is that rapidly growing fish are able to maximize their energy reserves prior to winter, when energy demand may outstrip supply (Schultz and Conover 1999).

Increased body size also has profound effects on the life history of juvenile salmonids. For resident salmonids such as rainbow trout or Dolly Varden char, increased size at age relates directly to earlier maturity (Stearns 1976; Thorpe 1990). Increased size among mature residents leads to increased fecundity (Stearns 1976). For anadromous fishes increased size influences migration timing and the size at which they smolt (Morgan et al. 2002; Thorpe 1990). Large smolts survive better at sea than small smolts (Henderson and Cass 1991; Jutila et al. 2006; Ward and Slaney 1988). For both life

history strategies increased size at the beginning of winter potentially leads to improved survival (Hurst 2007).

The influence of size on winter survival is summarized by the "critical size hypothesis". This hypothesis (Beamish and Mahnken 2001) states that the smallest individuals in a population are at risk of starvation when exogenous energy supplies are low. The combination of a hypoallometric relationship between size and metabolic rate and a hyperallometric relationship between size and energy storage is thought to result in starvation (Schultz and Conover 1999). The differing allometries mean that the disparity between metabolic demand and energy storage is most extreme for relatively small fish. This disparity accounts for reports describing size dependent mortality during winter (Toneys and Coble 1979). This effect of size on survival has also been reported for juvenile salmon in the marine environment (Beamish and Mahnken 2001; Moss et al. 2005) and in fresh water (Giannico and Hinch 2007; Quinn and Peterson 1996). Reduced size at the beginning of winter may represent an important bottleneck to freshwater production. Overwinter survival estimates for some salmonids species range as low as 15% in studies (Huusko et al. 2007).

The "critical size hypothesis" is predicated on the supposition that food supplies are low in winter, a supposition based on observations of decreased primary production during winter at high latitudes. Decreased food supplies have been used to account for energy loss among juvenile salmonids over winter (Berg and Bremset 1998; Finstad et al. 2004; Gardiner and Geddes 1980). However, food supplies available to juvenile salmonids have rarely been measured in winter. When food is available, juvenile

salmonids are capable of growing during winter (Cunjak and Power 1987; Giannico and Hinch 2007; Morgan et al. 2002), because juvenile salmonids are known to risk predation to forage when their energy reserves fall below critical levels (Bull et al. 1996; Metcalfe and Thorpe 1992). Starvation may therefore indirectly mediate size dependent mortality by placing small individuals at increased risk of predation.

While data describing prey availability to juvenile salmonids in winter are scarce, evidence suggests that foraging salmonids are likely to encounter prey. The densities (mg/m^3 stream water) of invertebrates in the winter drift of headwater streams of southeastern Alaska are highly variable but not seasonally dependent (Wipfli and Gregovich 2002). Similarly, the density of aquatic invertebrates found in riffles in Kennedy Creek, Washington does not differ between winter and summer (Honea 2005). While others have reported similar observations (Minikawa and Gara 1999), these studies were performed in relatively temperate streams and might not reflect northern latitude ecsoystems. Irons et al. (1993) characterized the winter behavior of aquatic insects in the interior of Alaska using laboratory and field studies, concluding that most insect taxa moved to unfrozen sections of the streambed in winter. These unfrozen sections included areas of groundwater upwelling and hyporheic zones. In winter, juvenile salmonids typically favor off channel habitats supplied by upwelling groundwater (Reynolds 1997, Huusko et al. 2007). Winter may therefore offer a concentrating effect on salmonid prey, placing them in the same locations sought by juvenile salmonids (Reynolds 1997, Huusko et al. 2007).

Increased size may have the added benefit of buffering juvenile salmonids against the deleterious effects associated with falling water temperatures. Acclimation to decreasing temperature is energetically demanding (Cunjak 1988; Reynolds 1997). Any increase in energy demand is likely to work against small individuals due to their reduced energy reserves. As stream temperature tends towards freezing it approaches the lower lethal limit for salmonids (Beitinger et al. 2004) and ion regulation becomes impaired (Hurst 2007). This thermal stress may be the underlying cause of size dependent mortality among juvenile coho salmon in British Columbia (Giannico and Hinch 2007). It is unclear if thermal stress exerts disproportionate effects on small individuals (Hurst 2007). The previously described winter preference for areas with upwelling groundwater likely helps juvenile salmonids avoid thermal stress (Reynolds 1997).

Goals for the rest of thesis

This thesis seeks to determine if the presence of salmon carcasses in fluvial habitats provides juvenile salmonids with energetic advantages toward surviving winter. The thesis proceeds along three fronts, each presented as a different chapter with separate goals. The goal of Chapter Two is to show how fatty acids can be used to discriminate energy sources and demonstrates that consumption of marine-derived lipids leads to increased energy stores in juvenile coho salmon and their prey. This chapter examines the energy reserves and fatty acid composition of consumers following exposure to different energy sources. In addition, the effect of lipid quality on fasting is examined. The results of those experimental manipulations are compared to observations made on coho salmon in natural streams before and after the arrival of adult carcasses. The goal of Chapter

Three is to understand how the nutritional state of juvenile coho salmon influences energy loss over winter. This chapter discusses the results of experiments designed to monitor changes in the body composition and energy content of fasting fish. The results from groups that started with different body compositions are related to observations made on overwintering coho salmon in a natural stream. The goal of Chapter Four is to determine how the findings of the first two chapters might relate to other species by examining the energy allocation strategies of a resident form of Dolly Varden char (*Salvelinus malma*). In this chapter, the hypothesis that the presence of carcasses improves the condition of consumers in fluvial habitats is considered by comparing seasonal changes in the growth and energy allocation of resident Dolly Varden char in streams with and without anadromous salmon populations. In aggregate, these chapters test the hypothesis that marine-derived lipids are integral to the winter survival of juvenile salmonids.

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Chapter II

Identification of Marine-Derived Lipids in Juvenile Coho Salmon (*Oncorhynchus kistuch*) and Aquatic Insects Using Fatty Acid Analysis[1]

Abstract

The energetic benefits enjoyed by consumers in streams with salmon runs depend on to how those benefits are accrued. Adult salmon deliver significant amounts on nutrients (i.e. nitrogen and phosphorous) and tissue to streams when they spawn and die, which can have demonstrable effects on primary production in streams. Benefits to consumers derived from improved bottom-up production are likely to become more diffuse as the trophic distance between producers and consumers increases. In contrast, consumption of carcass tissues and /or eggs provides for direct energy subsidies to consumers. In this study, comparisons of coho salmon (*Oncorhynchus kisutch*) and aquatic insects exposed to terrestrial and marine energy sources demonstrated that direct consumption of marine-derived lipids has a significant effect on the lipid reserves of consumers. Aquatic insects and coho salmon were reared for six weeks in artificial streams supplied with terrestrial or marine energy sources. Chironomids, nemourids and juvenile coho salmon exposed to the marine energy source altered their fatty acid compositions by incorporating long-chain polyunsaturated fatty acids characteristic of marine fish. Baetid mayflies were unaffected. The direct movement of specific fatty

[1] Heintz, R.A., M. S. Wipfli and J. P. Hudson. Identification of marine derived lipids in juvenile coho salmon (*Oncorhynchus kisutch*) and aquatic insects using fatty acid analysis. Prepared for submission in Transactions of the American Fisheries Society.

markers indicated that changes in fatty acid composition resulted from direct consumption of marine-derived lipids. In addition, these same consumers had significantly more lipid and triacylglycerols than those in artificial streams supplied with terrestrial energy sources. Similar results were obtained from coho salmon sampled from natural streams before and after the arrival of adult salmon runs. These data indicate that marine-derived lipids are an important source of reserve lipids for consumers that overwinter in streams.

Introduction

Identification of the energy sources fueling consumers in oligotrophic streams is important to understanding the role that marine-derived nutrients play in stream food webs. Coastal streams in Alaska and the northeast Pacific are typically oligotrophic due to reduced availability of nitrogen, phosphorous (Ashley and Slaney 1997) or carbon (Wipfli et al. 2004). However, most of these streams receive significant nutrient subsidies in the form of adult salmon carcasses each year. Returning adult salmon supply streams with nutrients (i.e. N and P) and tissues when they spawn and die. Nutrient subsidies can lead to increased primary production (Johnston et al. 2004) and biofilm mass (Wipfli et al. 1998) which may increase productivity through bottom-up processes. However the effects of these subsidies on the biomass of producers can vary significantly from year to year within a stream (Mitchell and Lamberti 2005). Carcass tissues, such as eggs and flesh, may provide consumers with direct energy subsidies in the form of reduced carbon packaged as carbohydrate, lipid and protein. The nutrient and energy subsidies offered by spawning adult salmon can lead to increases in the density of aquatic insects (Wipfli et al. 1999) and in the growth of juvenile salmonids (Giannico and Hinch 2007; Wipfli et al. 2003; Wipfli et al. 2004). However, the relative importance of bottom-up processes versus direct consumption is unknown.

It is likely that the benefits enjoyed by consumers in streams receiving salmon carcasses relate directly to how those benefits are accrued. Marine-derived nutrients can potentially boost productivity and improve growing conditions for consumers through bottom-up processes (Johnston et al. 2004). However, residence time for salmon

carcasses can be very short (days) in small streams (Gende et al. 2004), limiting the potential for amplification of bottom-up production. In contrast, the nutritional benefits derived from ingesting marine-derived tissues may be substantial; rainbow trout (*Oncorhynchus mykiss*) increased their daily consumption rates by more than 400% after sockeye salmon (*O. nerka*) returned to spawn (Scheurell et al. 2007). These data suggest that the value of marine subsidies to consumers is likely greatest when the consumers have direct access to marine-derived energy.

Methods used to identify the energy sources used by consumers have traditionally been limited to stomach content and stable isotope analyses. The ultimate sources of energy in most ecosystems are primary producers that reduce carbon via photosynthesis. Ultimate energy sources to streams can be freshwater algae or terrestrial plants. Marine algae can also be an ultimate energy source in streams with anadromous fishes, because spawning adults deliver carbon reduced by marine algae. Stomach content analysis can only identify what consumers have most recently eaten, providing limited information about the ultimate source of energy. Stable isotope analysis has been useful in identifying the carbon source consumed by stream-dwelling organisms (Bilby et al. 1998; Chaloner et al. 2002; Hicks et al. 2005; Kline et al. 1990). However, stable isotope analysis provides little information on the nutritional condition of consumers. If the goal of source identification is to understand the ecological importance of different energy sources then identification should be coupled with methods for measuring consumer condition such as energy reserves or lipid content.

Fatty acid analysis is a method that can be used to identify energy sources that also provides information on consumer condition. The fatty acid compositions of consumers are determined by their diets because ingested fatty acids are stored in reserve lipids with little or no modification when ingested fat is surplus to immediate metabolic needs (Budge et al. 2006). Therefore, fatty acid analysis can be used to identify energy sources when the putative sources have distinct fatty acid compositions. For example, the well known differences in the fatty acid compositions of marine and freshwater fishes (Henderson and Tocher 1987) have been used to infer the energy sources consumed by stream fauna (Heintz et al. 2004; Volk 2004). Variations in lipid content among groups indicate variations in the nutritional status of the groups. Hence, fatty acid analysis provides measures of the nutritional state of the consumer while simultaneously providing information of the source of nutrition.

Despite the apparent utility of fatty acid analysis, the transfer of marine fatty acids from carcasses to juvenile salmonids has not been directly demonstrated. A direct demonstration requires showing that the presence of carcasses induces a shift in fatty acid composition that does not otherwise occur. There is some evidence these shifts occur. Fish and insects in streams that receive carcasses have higher amounts of the fatty acids typical of marine species than those in streams without carcasses (Volk 2004). Similarly, coho salmon (*O. kisutch*) in artificial streams loaded with carcass tissue can have higher amounts of fatty acids associated with marine species and greater energy reserves (Heintz et al. 2004). Neither of these studies documented diet shifts or attempted to identify the fatty acid composition of consumers prior to the arrival of carcasses. Demonstration of

the transfer of marine fatty acids from salmon carcasses to consumers would indicate that the mechanism of transfer is through direct consumption; offering the opportunity to identify the source of energy, the mechanism of acquisition, and its nutritional value.

The objectives of this study are to determine if fatty acids delivered by adult salmon are directly transferred to consumers and to determine if that transfer improves consumer condition. The movement of marine-derived lipids and the consequences of this movement were monitored in juvenile coho salmon and aquatic insects under controlled conditions in artificial streams. To examine the transfer of marine-derived fatty acids, coho salmon and insects were maintained in artificial streams supplied with different energy sources for six weeks. The fatty acid compositions of fish and insects at the end of the study were compared with the compositions of the different energy sources to determine if source fatty acids were transferred to consumers. The condition of the consumers was simultaneously determined by measuring their lipid content and the proportion of that lipid allocated to lipid reserves (i.e. triacylglycerols). Finally, wild fish were sampled from natural streams before and after the arrival of adult salmon carcasses to determine if changes observed under controlled conditions could also be observed in the field.

Methods

I combined laboratory and field studies near Juneau, Alaska (58°37′16′′N; 134°56′11′′W) to test the hypothesis that consumers exposed to marine-derived lipids develop improved nutritional condition by directly consuming carcass tissues. The laboratory study, located near Sheep Creek (Figure 2.1) involved artificial streams

(mesocosms) supplied with different energy sources: marine, unenhanced and enhanced terrestrial sources. Consumers in the streams consisted of aquatic insects and coho salmon. The mesocosm operated between 23 July and 4 September, 2001. At the end of the study, insects and fish were removed and analyzed to determine their total lipid, lipid class, and fatty acid composition. Total lipid and lipid class were used to determine the nutritional status of the organisms, and fatty acids were compared to those of the ingested energy sources.

The field study focused on coho salmon collected from three natural streams (Figure 2.1). Fish were collected in July before the arrival of adult chum (*O. keta*) and pink salmon (*O. gorbuscha*) and in October, after spawning was complete. Fatty acid compositions were compared to those of salmon carcasses to verify the results of the artificial stream study.

Mesocosm experiment

The mesocosm consisted of a set of parallel artificial streams supplied with water from above a natural barrier to immigration. The mesocosm was built alongside Sheep Creek (Figure 2.1) and has been previously described in detail (Wipfli et al. 2004). Briefly, the artificial streams were arrayed on each of six tables so that there was one channel of each treatment per table. Each channel was constructed from plywood (294 x 18 x 23 cm deep), divided into three sections: an upper pool (102 x 18 cm), a riffle (66 x 18 cm) and lower pool (118 x 18 cm). Water entering the upper pool flowed (0.6 L • minute $^{-1}$) over the riffles and out the lower pool, allowing invertebrates to pass through and colonize the riffles. Water temperatures ranged between 6.2 and 9.3 °C during the

study. The lower pool in each mesocosm received 4 coho salmon captured from a nearby stream: a small (0.5 ± 0.1 g), small-medium (0.8 ± 0.1 g), large-medium (1.12 ± 0.2 g) and large (1.74 ± 0.4 g) fish. Only the two largest fish were processed for lipid analysis .

Each of the channels on a table received a different treatment following a randomized block design where the tables were the blocks and each of the treatments was randomly assigned to a channel on the table. Treatments were placed in the upper pool. The treatments included no treatment (control), fertilizer, and carcass tissue. They were intended to represent the natural terrestrial, enhanced terrestrial and marine-derived energy sources, respectively. The terrestrial energy source was expected to reflect the combined autochthonous and allochthonous sources found in a typical stream without nutrient subsidies (i.e. Upper Sheep Creek). The fertilizer treatment was expected to enhance the production of autochthonous energy sources and represent a stream receiving nutrient subsidies except for carbon. The carcass tissue treatment was expected to simulate the effects of marine-derived energy. Fertilizer (16-30-0 N-P-K) consisted of slow-release nutrient pellets (8 g) manufactured by Lesco, Inc. Fertilizer contained 16.0% nitrogen as urea (4.0%) and magnesium ammonium phosphate (12.0%), phosphoric acid (30.0%, 13.1% as P), magnesium (11.0%), and vegetable oil (2.0%). Carcass treatments consisted of eggs and soma taken from female chum salmon (*O. keta*) that had returned to Lower Sheep Creek. Carcass and fertilizer applications were intended to supply equal amounts of phosphorous to the water. The fertilizer and carcass treatments described here released 10.3 and 9.3 g of phosphorous, respectively over the six weeks of the

experiment. A total 3.8 g of phosphorous passed into the control channels (unpublished data).

The mesocosm experiment continued for six weeks. Water was first introduced into the mesocosm on 16 July and the experiment continued until 4, September, 2001. A week after the water began flowing, the channels were treated and fish were introduced two to three days later. After six weeks, the fish were collected from the channels, measured, and frozen for analysis. Insects in the families Chironomidae (midge), Baetidae (mayfly, primarily *Baetis sp.*) and Nemouridae (stonefly, primarily *Zapada sp.*), the most dominant taxa found in the mesocosm, were collected from the channels and frozen. In addition, samples of chum salmon egg and soma were retained for analysis as were samples of coho salmon at the start of the experiment. All samples were maintained whole at -80 °C for approximately four months until they were processed.

Natural stream sampling

Coho salmon were collected from Bridget Cove Creek, Peterson Creek and Fish Creek (Figure 2.1) before and after the arrival of adult salmon. The streams, typical of salmon streams in the region, are fed by surface and groundwater bordered by dense spruce (*Picea sitchensis*) and hemlock (*Tsuga herterophylla*) forests. However, as each stream nears saltwater the spruce-hemlock canopy gives way to red alder (*Alnus rubra*) on beach fringes and ultimately the streams cut through beach marsh. The portions of the streams receiving adult salmon are relatively short, and substrates consist of granitic cobbles and washed glacial debris. Precipitation in the region averages 150-500 cm/year. Each of the streams receives adult pink (*Oncorhynchus gorbuscha*), chum

(*Oncorhynchus keta*) and coho salmon. Foot surveys in 2001 determined the mean spawner density of adults from all species to be 0.04 and 0.07 spawners/m^2 in Fish and Peterson Creeks, respectively (Mitchell and Lamberti 2005). Adult chum and pink salmon represent the greatest source of carcass biomass in these streams. Adult salmon arrive between July and September (Halupka et al. 1996).

Coho salmon were collected with minnow traps baited with salmon eggs from each of these streams before and after the arrival of adult salmon. Traps were set for 24 h in multiple locations throughout the portions of these streams accessible to anadromous adults. Fish collected between 29 June and 12 July, 2001 are hereafter referred to as those collected before adults returned. A subsequent collection of fish, made between 20 September and 2 October, 2001, is hereafter referred to as those made after adults returned. In addition, fish were collected from Peterson Creek on 18 April, 2001. Fish that over winter in streams rely on energy stores to survive winter (Post and Parkinson 2001) and resume foraging in spring. The April samples thus provided an opportunity to determine if the marine-derived fatty acids are retained over winter. Between five and twelve fish were retained from each sampling period and location for lipid analysis. The wet mass of each fish was recorded. Fish were frozen whole at -80 °C for six to nine months until they were processed.

Lipid extraction

Lipid was extracted from homogenized samples of wet tissue using modifications of Folch et al.'s (1957) method, depending on the taxon sampled. Lipids from fish were

extracted using a Dionex Accelerated Solvent Extractor (ASE) 200®[2] with 2:1 (v:v) chloroform:methanol. Fish were homogenized using a mortar and pestle and 0.5 and 1.0 g samples of homogenate were placed in a beaker, coarsely mixed with diatomaceous earth to absorb water and then loaded into ASE cells along with 8 g sand. Extractions were run at 120 °C and 500 psi. Extracted lipid was stored in vials loaded with chloroform and 0.01% butylhydroxytoluene (BHT) to prevent oxidation. Insects from a mesocom were combined by taxon into a sample weighing between 8 and 150 mg, blotted dry, placed in a glass tube with 100 µl solvent (chloroform:methanol 2:1 vol/vol) and homogenized with a glass pestle. The mixture was passed through a pre-wetted filter paper to remove particulates and the volume adjusted to 1.0 mL. All extractions were performed immediately after homogenization.

All lipid extracts obtained from the ASE were purified following the same procedure. Extracts were washed successively with a 0.88% KCl solution and 1:1 (v:v) methanol:deionized water in a volume equal to 25% of the extract volume to remove water and coextractables. Excess solvent was evaporated to 1.0 mL. The percent lipid (wet mass basis) in all extracts was calculated gravimetrically from a 500 µl aliquot. Water content of the insects was considered negligible. Quality assurance samples included with each batch of 1 5 samples included a blank to control for cross contamination, and a duplicate reference material to evaluate repeatability. The reference material was retained with the batch throughout all subsequent analyses. Reference lipid

[2] Use of trademarks does not imply endorsement by the National Marine Fisheries Service.

content was always within 10% of the expected value, and coefficients of variation for the duplicated samples averaged 5.6%.

Lipid class separations

The triacylglycerols (TAG) of coho and chironomids were isolated by high performance liquid chromatography (HPLC). The HPLC method (Christie 1985) allowed for resolution and quantification of the contributions of wax and cholesterol esters (WE-CE), sterols (ST), mono-acylglycerols, free fatty acids (FFA) and five phospholipids to the total lipid. The phosphoslipids included phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), sphingomylin (SPH) and lyso-phosphatidylcholine.(lyso-PC) The HPLC system consisted of a Hewlett Packard Series 1050 quaternary pump, Gilson 231XL injector, Phenomonex Sphereclone® 3 μm silica column, Foxy 200 fraction collector and a Sedex 55 evaporative light scattering detector (ELSD). The method employed a solvent gradient consisting of hexane/chloroform (1:1) at injection, followed by iso-octane/tetrahydrofuran (99:1), isopropanol/chloroform (4:1) and isopropanol/water (1:1) over a 30 minute run time. A proportioning valve split the TAG fractions (60:40) between ELSD and a collection vial. Toluene and 0.01% BHT were added to vial and the volume reduced to 1 ml under a stream of nitrogen. The time at which fractions were collected was set after viewing the retention time of TAG in the calibration standards at the start of each sample run. When the run was completed, TAG fractions were topped with nitrogen and stored at -20°C until transesterification.

Lipid class concentrations were determined from three point calibration curves using calibration standards normalized to an internal standard (1,2-dipalmitoyl-sn-

glycero-3-phosphoethanolamine-N,N-dimethyl). Peaks were identified by comparing their retention times with those of calibration standards. The calibration standards (in order of elution) included myristic acid, triolein, cholesterol, monolein, C21:0, plant phosphatidylinositol, egg phosphatidylethanolamine and bovine phosphatidylserine and choline. Quality assurance samples included with each batch of 15 samples included a method blank, the previously described duplicated reference materials, and two of the calibration standards. The coefficient of variation for a given lipid class in the reference material averaged 10%. Masses recovered from the calibrations standards averaged 97% of the predicted values. For the samples, an average (± 1 s.e.) 954 ± 23 mg of lipid was recovered for every gram of lipid injected. Concentrations of the classes are expressed as the percent of the total mass recovered.

Fatty acid composition of lipids and triacylglycerols

Triacylglycerols collected from chironomids and coho salmon and purified whole lipids from chum salmon tissues and all the insects (Table 2.3) were transesterified to fatty acid methyl esters (FAMEs) using Hilditch reagent as described by Christie (1982). The entire TAG fraction or 300 μg of lipid was added to 1 mL hexane and spiked with 19:0[3] and 23:0. This was mixed with 2 mL of a 1.5% solution of H_2SO_4 in methanol (v:v) and then heated at 80°C for 2 hours. Five mL of a 5% NaCl solution (v:v) were added during cooling, and the cooled solution was extracted twice with 4 mL of hexane. Each

[3] Fatty acid names follow the convention X:YnZ where X refers to the number of carbons, Y the number of double bonds and Z the location of the first double bond counting from the methyl end. Co-eluting peaks that cannot be resolved are identified as X:YnZ+Z', where Z' refers to the location of the first double bond in the co-eluted peak.

time the extract was washed with 2% $KHCO_3$ (m:m) and dried over Na_2SO_4. The purified FAMEs were collected in a clean vial, and the volume adjusted under nitrogen to 1 mL. The internal standard (C21:0) was added to the vials, which were stored under nitrogen and stored at -20° C until they were injected into the gas chromatograph.

The concentrations of 32 fatty acids were measured using temperature programmed gas chromatography and mass spectrophotometry (GC/MS). The FAMEs were separated on a Hewlett Packard model 6890 GC using a 30 meter Omegawax 250 μ fused silica capillary column. Fatty acid masses were measured with a Hewlett Packard model 5973 equipped with a mass selective detector (MSD) operating in selected ion monitoring mode. Fatty acid concentrations were determined using a five point FAME calibration curve normalized to surrogates (19:0 and 23:0) added prior to transesterification. The calibration curves were developed each day that samples were analyzed. Fatty acid concentrations were corrected to reflect the molecular weight differences between the FAME and the respective acid. Concentrations were further corrected by subtracting the concentrations for each fatty acid observed in method blanks. Recovery rates of the surrogates were estimated by comparing observed masses to masses of the internal standard (21:0). The recovery of the surrogates averaged 95.7 ± 1.1%. Estimates of analytical error determined from reference samples processed at the same time as the samples averaged 13 ± 1.0%. Fatty acid concentrations are reported as percentages of the total mass of fatty acids observed.

Data reduction and analysis

Statistical analyses were designed to determine the effects of carcasses on insect and fish lipids in the mesocosm. Measurements taken from coho salmon in the mesocosm, including weight, percent lipid, TAG and structural lipid were examined by a two-way ANOVA with energy source as the treatment and table as the blocking variable using the following model.

$$R = E + T + E \times T \qquad \text{(Equation 2.1)}$$

Where R is the response (length, lipid, TAG or structural lipid), E is the energy source, T is the table the channel was on and E×T their interaction. E was considered a fixed factor and T a random factor. Percent TAG was estimated as the contribution of TAG to total lipid mass recovered during lipid class analysis. The percent structural lipid was estimated as the summed contribution of sterols and phospholipids to the total lipid mass. Observations on the insects included weights and percent lipid. ANOVAs were similar to those in equation (2.1), but the interaction term was not tested, because no replicate samples were available for individual channels. In addition, TAG and structural lipid were tested for the chironomids. Observations made on fish collected from wild streams before and after adult runs were compared by a nested ANOVA with time as a fixed factor and streams as random factors nested in time:

$$R = T + S(T) \qquad \text{(Equation 2.2)}$$

Contrasts included comparisons of fish collected before and after adult returns in each stream. For the Peterson Creek samples the spring data set was also included. This led to 5 pairwise *post hoc* comparisons, thus significance was assessed at $\alpha = 0.01$.

Multivariate analyses of the fatty acid compositions of different test groups relied on non-parametric multi-dimensional scaling (NMDS). Three separate models were developed: one for coho salmon from the mesocosm experiment, the second for the insects from the mesocosm experiments and the third for the coho salmon from the natural streams. Chum salmon tissues were included in each of the models as well as coho salmon collected prior to the beginning of the mesocosm experiment. These latter fish are hereafter referred to as the "initial coho salmon". For each model, relationships between test groups were examined in spaces ranging from 1 to 5 dimensions to determine the optimal space for resolving the groups. Models were constructed from a distance matrix, $\boldsymbol{D}$, where each element, D_{ij} was the distance between two samples. The distance, $D(\mathbf{A},\mathbf{B})$, between the A^{th} and B^{th}, samples was found by:

$$D(\mathbf{A},\mathbf{B}) = \sqrt{\sum_{i<j}\left(\log\frac{\mathbf{A}_i}{\mathbf{A}_j} - \log\frac{\mathbf{B}_i}{\mathbf{B}_j}\right)^2} \qquad \text{(Equation 2.3)}$$

A and **B** are vectors containing the fatty acid compositions of two animals. The set of fatty acids used in constructing a matrix was limited to those that were observed in all of

the animals in a given set. In some cases it was necessary to delete animals from the analysis when they had a relatively large number of undetected fatty acids due to small TAG or lipid masses prior to transesterification. Once a set of fatty acids was selected, the values were normalized to unit sum prior to calculating ***D***. The best description of the data set in reduced space was determined by examining Shephard diagrams and evaluating the relationship between Kruskal's stress and the number of dimensions in the model (Clarke and Warwick 1994).

The potential energy sources consumed by coho salmon in the channels were represented by the initially sampled coho salmon and the carcass tissues. Chum salmon returning to freshwater represent a discrete energy source. In contrast, the terrestrial and freshwater energy sources supplied to the channels are diverse and the relative contributions of each source to a consumer cannot be reliably determined. However, the tissues of coho salmon sampled prior to the experiment integrated all of the potential terrestrial sources into a discrete source. Similarly, I used the compositions of the insects collected from the control channels as the discrete indicator of the terrestrial energy source for each of the insect taxa examined.

Analysis of similarity (ANOSIM) was used to test the hypothesis that the fatty acid compositions of animals from a given treatment differed from those of the same species undergoing alternative treatments (Clarke and Warwick 1994). The chum salmon tissues and initial coho salmon were excluded from these analyses. In the event that the null hypothesis was rejected, *ad hoc* comparisons were made between each pair of treatments with α set equal to α / n where n was the number of *ad hoc* comparisons. The

relatively small number of observations of insects prevented *ad hoc* comparisons between treatments. Similarly, comparisons among coho salmon from the wild streams were limited to contrasts between the fish collected before and after adult returns in a given stream. In addition, coho collected in April from Peterson Creek were contrasted with those collected before and after adult returns.

Results

Effect of carcasses on nutritional condition of coho in artificial streams

Coho salmon living in artificial streams subsidized with carcasses were heavier and had twice the lipid stores of coho salmon in the other streams. The wet mass of coho salmon from the carcass-treated channels was approximately one third greater than that of either of the other treatments ($F_{2,18} = 30.67$; $P < 0.001$). Coho salmon from the artificial streams differed in their lipid content and the proportion of lipid allocated to storage (i.e. TAG) ($F_{2,18} > 23.59$; $P < 0.001$) (Table 2.2). These differences in lipid were consistent with differences in the proportion of lipid allocated to storage. Lipids in coho from the carcass-treated channels had more than twice the amount of TAG as lipids in the initial fish and those from the control and fertilizer-treated channels, respectively (Table 2.2). In contrast, coho salmon in carcass-treated channels had reduced amounts of lipid allocated to structural lipid classes relative to those in control or fertilizer-treated channels ($F_{2,18} = 50.72$; $P < 0.001$) (Table 2.2).

Effects of carcasses on nutritional condition of insects in artificial streams

The effect of carcasses on nutritional condition of stream insects differed among species. Neither the size nor lipid content (Table 2.2) of baetids or nemourids depended on energy source ($F_{2,5} < 4.21$; $P > 0.085$). However, nemourids in the carcass-treated channels averaged 50% more lipid ($P = 0.085$) than those in the other channels. A similar and statistically significant effect was observed among the chironomids. In the carcass-treated channels, chironomids were at least 25% heavier and 50% fattier than those in the control or fertilizer-treated channels. While the weight difference was significant (($F_{2,6} = 11.03$; $P = 0.010$) there was no detectable difference in percent lipid ($F_{2,6} = 3.11$; $P = 0.119$). However, chironomids in the carcass-treated channels had significantly more lipid allocated to TAG ($F_{2,3} = 23.72$; $P = 0.015$) (Table 2.3). Conversely, chironomids in carcass-treated channels had significantly less lipid allocated to structural classes than those in other channels ($F_{2,3} = 70.06$; $P = 0.003$).

Effect of carcasses on nutritional condition of coho salmon in natural streams

Increased lipid reserves in juvenile coho salmon coincided with the arrival of carcasses in natural streams. Lipid increased significantly between early July and late September in coho salmon from each of the three streams examined ($F_{4,43} = 21.92$; ,$P < 0.001$). Average lipid content increased two to threefold in Bridget Cove, Peterson Creeks and Fish Creek (Table 2.3). Similarly, coho salmon increased the proportion of lipid allocated to TAG ($F_{4,43} = 11.38$; $P < 0.001$) after adults arrived. TAG levels increased at least a threefold when it was expressed on a wet mass basis. Increased

allocations to TAG coincided with significant decreases in the proportion of lipid allocated to structure ($F_{4,43} = 14.50$; $P < 0.001$).

Fatty acid compositions of putative energy sources

The fatty acid compositions of the insects collected from the control channels were dominated by saturated and mono-unsaturated fatty acids. These two classes accounted for 65% to 71% of the total fatty acids recovered from nemourids and chironomids, respectively (Table 2.4). Saturated fatty acids were the more abundant of the two. The most abundant polyunsaturated fatty acids (PUFAs) were those with 18 carbons, particularly 18:3n3 and 18:2n6. Concentrations of long-chain PUFAs (20 and 22 carbons) were low; accounting for less than 15% of the total mass of fatty acids.

Fatty acid compositions of coho salmon collected at the beginning of the mesocosm experiment were qualitatively similar to those of the insects with some notable differences. Like the insects, saturated and mono-unsaturated fatty acids accounted for nearly 70% of the total fatty acid mass detected (Table 2.5). In contrast to the insects, mono-unsaturated fatty acids were the more abundant of the two fatty acids. Similar to the insects, the most abundant PUFAs were those with 18 carbons and long-chain PUFAs were much less abundant. However, coho salmon had more 22:6n3 than the insects.

Fatty acid compositions of the chum salmon tissues were notably different from those of the coho salmon and insects. While saturated and mono-unsaturated fatty acids were still the most abundant classes, they accounted for less than 60% of the total mass because there were much greater amounts of PUFAs (Table 2.5). Among the mono-unsaturated fatty acids, chum salmon soma had conspicuous amounts of 20:1n9+11and

C22:1n9+11. The relative abundance of these fatty acids was tenfold more than that of the initial coho salmon or the insects. Unlike the samples collected from freshwater, long-chain PUFAs dominated the PUFAs, particularly 22:6n3, which accounted for more than 20% of all the fatty acids observed. The 18 carbon PUFAs accounted for less than 4% of the total mass.

Transfer of marine-derived fatty acids in artificial streams

The presence of carcasses had a significant effect on the fatty acid compositions of coho salmon in the mesocosm. Fatty acid compositions of coho salmon in the different channels differed significantly at the end of the experiment ($R = 0.516$; $P = 0.001$) (Table 2.6). The plot of the two-dimensional NMDS model (Figure 2.2) revealed a distinct separation of the chum and coho salmon fatty acid compositions. Compositions in coho salmon from carcass-treated mesocosms bore a greater similarity to those of the chum salmon than any of the other groups. In contrast, coho collected from the other channels had similar fatty acid compositions to the coho salmon sampled at the beginning of the experiment. These observations were supported by *ad hoc* comparisons using ANOSIM. The fatty acid compositions of carcass-treated coho salmon were dissimilar to those of control or fertilizer-treated coho salmon ($R > 0.72$; $P < 0.001$). In contrast, compositions of coho salmon from control and fertilizer-treated channels were indistinguishable ($R = 0.00$; $P = 0.421$).

Similar to coho salmon, chironomids and nemourids in carcass-treated channels acquired marine-derived fatty acids. Tests (ANOSIM) comparing chironomid and nemourid fatty acid compositions indicated differences among treatments ($R > 0.606$; $P <$

0.004), but baetids were unaffected (R = -0.039 P = 0.527). NMDS plots (Figure 2.3) indicated that differences among treatments for the former two species were related to the presence of carcasses.

Transfer of marine-derived fatty acids to coho salmon in natural streams

A change in the fatty acid composition of juvenile coho salmon coincided with the arrival of adult salmon in the natural streams. ANOSIM tests indicated significant differences in the fatty acid compositions of coho salmon collected before and after the arrival of adult salmon in Peterson Creek (R = 0.544 ; P =0.026), Fish Creek (R = 0.517; P =0.013) and Bridget Cove Creek (R = 0.866; $P < 0.001$). NMDS plots indicated that these differences resulted from increased similarity to chum salmon fatty acids among coho salmon collected after adults arrived (Figure 2.4). Samples collected in spring from Peterson Creek appeared to be located intermediate to those collected before and after adult salmon returned. Comparisons by ANOSIM indicated that spring samples did not differ from those collected before adults arrived (R = 0.281; P = 0.104), but they were different from those collected after adults arrived (R = 0.712; P = 0.009).

Discussion

The delivery of marine-derived carbon into streams by adult salmon provided measurable nutritional benefits to high-level consumers. Coho salmon and chironomids in carcass-treated channels were larger and had an increased supply of stored lipids. Increases in storage lipid (triacylglycerides) in both species resulted from the incorporation of long-chain PUFAs derived from carcass tissues, which contributed to a

greater similarity between freshwater consumers and carcass tissues. These effects were consistent with observations made on coho salmon in natural streams, suggesting that carcasses play an important role in provisioning juvenile salmonids prior to winter.

Incorporation of marine-derived fatty acids without modification indicates the observed increases in size and energy reserves derived from direct consumption of marine lipids. In this case, direct consumption means marine-derived lipids became available to consumers without the modifications that normally accompany decomposition of carcass tissues and lipid oxidation. Hence, lipids became available either by direct consumption of carcass tissues or consumption of consumers that ingested carcass tissues. The fatty acid data and the availability of marine lipids relative to other forage are discussed later in relation to the conclusion that direct consumption of marine lipids leads to increased size and energy stores in stream consumers.

Evidence for direct consumption of marine-derived lipids

The fatty acid compositions of the putative energy sources described here highlight the flow of marine-derived lipids from carcasses into consumers. Some of the fatty acids observed in chum salmon tissues had limited availability in the freshwater systems studied. These included, 22:6n3, 22:5n3, 20:1n9+11 and 22:1n9+11. The relative concentrations of these fatty acids were higher in carcass-treated fish, nemourids, and chironomids compared with those from the other channels. Similarly, the concentrations of these fatty acids increased in coho salmon from the natural streams after the arrival of carcasses. The latter mono-unsaturated fatty acids likely derive from the alcohols of calanoid copepod wax esters (Saito and Kotani 2000). Marine flagellates are an important

source of 22:6n3 (Napolitano 1999), and 22:5n3 is an intermediate step in the synthesis of 22:6n3 (Guschina and Harwood 2006). In contrast, diatoms, particularly those in periphyton, have low levels of the long-chain PUFAs, but they produce high levels of 18:3n3 and 18:2n6 (Napolitano 1999). Similarly, hemlock and alder have higher amounts of 18-carbon PUFAs relative to long-chain PUFAs (Volk 2004).

The altered fatty acid compositions of the coho salmon in the carcass-treated channels and in natural streams were consistent with those of coho salmon fed diets containing fish oils. Coho salmon fed a diet consisting of 12% fish oil had twice the amount of 22:6n3, and nearly half the amount of 18:2n6, as those fed a 12% beef tallow diet (Yu and Sinnhuber 1981). Marine fatty acids were readily acquired by coho salmon studied here. These may have come from consumption of carcass tissues, chironomids, or nemourids. Insect prey from carcass-treated mesocosms had about sevenfold more 22:6n3 than those in the control and fertilizer-treated mesocosms, and about one third the 18:2n6.

In contrast, it is likely that relatively low levels of PUFAs in consumers from the control and fertilizer-treated channels resulted from feeding on terrestrial energy sources. Producers in riparian habitats also have low levels of PUFAs. Volk (2004) found PUFAs comprised no more than 26 to 33% of fatty acids observed in hemlock and alder, respectively and 26% in stream periphyton. PUFAs in these producers were primarily 18 carbon PUFAs , similar to the consumers in control and fertilizer-treated channels. Moreover, PUFA levels in periphyton have been found to correlate with those of grazers, including baetids (Volk 2004). Goedkoop et al. (2007) reported a near perfect correlation

between dietary PUFA levels and the levels observed in *Chironomus* larvae, indicating that chironomids retain dietary PUFA with little modification to their relative proportions at ingestion.

Relative availability of marine-derived lipids to consumers

The mass of marine-derived lipid relative to that of other dietary items accounts for the changes in fatty acid composition of the consumers in the carcass-treated channels. Chum salmon soma averaged 1.8% lipid and eggs 10.7% lipid while the lipid content among benthic invertebrates ranged between 4.5 and 6.7%. The combined average lipid content of the carcass tissues was therefore proportionate to that of the insects. In contrast, the amount of marine-derived lipid far outweighed that of the insects. Combining the counts of insects in the carcass-treated channels (unpublished data) with average insect mass (Table 2.3) indicates that the biomass of the carcass tissue was more than 3000 times that of the insects. Probably only a fraction of that biomass was available to the coho salmon, but it is clear that the mass of insects never approached that of the carcass tissues. Even if the entire mass of all insects found in the channels turned over on a daily basis, the total mass over the 40 d experiment could not have approached that of the carcasses. It is unlikely that such a turnover occurred for chironomids and nemourids, because they also took on marine-derived lipids. The effect of this apparent flood of food on coho salmon is consistent with the idea that the ratio of subsidy to comparable ambient resources predicts the effect of the subsidy, particularly for generalists such as coho salmon (Marczak et al. 2007). Thus, a similar situation likely existed for the coho salmon in the natural streams.

Increased food availability in the carcass-treated channels is also indicated by the increased size and lipid reserves in consumers. The increased size of the coho salmon suggests they had enhanced growth during the study. Similar effects of marine subsidies on lipid and growth were demonstrated in stream-dwelling coho salmon supplemented with frozen euphausids (Mason 1976). In other experiments coho salmon in carcass-treated channels had higher growth (Wipfli et al. 2003) and lipid content (Heintz et al. 2004) than those in control channels, as did juveniles in treated stream reaches (Wipfli et al. 2003; Wipfli et al. 2004) and coho salmon in beaver ponds with naturally occurring carcasses (Lang et al. 2006). In juvenile rainbow trout, the allocation of ingested energy to lipid reserves depends on growth such that slower-growing individuals allocate all of their energy to structure (Post and Parkinson 2001). Increases in mass among slower growing individuals were almost entirely allocated to protein and structural lipids in that study. Here, coho salmon in the carcass-treated channels increased their mass by 60% and doubled their TAG content. Those in the other channels increased their mass by 20% and did not change their TAG content. Fish in Bridget Cove had the same initial size as those in the channels and increased their mass by 80% while more than doubling their TAG content after carcasses arrived.

Similarly, the relatively large mass of carcass tissue introduced into the channels accounts for the improved nutrition among nemourids and chironomids. *Zapada sp.* are trophic generalists that shreds and then consumes periphyton and coarse organic detritus (Minhuc and Minhuc 1995), was the most abundant of the nemourids sampled. It is possible that chironomids and nemourids could have benefited from increased periphyton

production, but changes in their fatty acid compositions indicate that carcass tissues were a more important source of nutrition. The mass and residence time of carcass-derived particulates in the mesocosms is unknown, but they could have settled in the pool occupied by the coho salmon, chironomids and nemourids. This availability of carcass-derived particulates is consistent with observations of increased size and density of the chironomids in the carcass-treated channels and the idea that food quality and quantity influences chironomid growth (Goedkoop et al. 2007). This also explains the lack of response by baetids, which were more likely to drift through the mesocosms without taking up residence. In previous studies, baetids did not colonize carcass tissues (Chaloner and Wipfli 2002; Wipfli et al. 1998), indicating that they would most likely accrue benefits from carcasses through bottom up processes.

Direct consumption of marine lipids by consumers is further supported by other reports. Observations of increased density of coho salmon (Bilby et al. 1998) and chironomids (Lessard and Merritt 2006) following the arrival of carcasses suggest carcasses are recognized by consumers as a food source. Johnston et al. (2004) reported that marine-derived carbon was the primary source of particulate organic carbon input into streams in British Columbia. The increased density of coho salmon reported by Bilby et al. (1998) was likely a functional response resulting from their mobility and ability to detect food sources at a distance (Eastman 1996). Increased density of chironomids could also be a functional response on the part of localized populations that drift into areas of high food availability.

Relevance of these data to existing literature

These data add to the growing body of literature describing the effects of salmon carcasses on consumers by quantifying the nutritional benefits to fish and aquatic insects. Previous work describing the effects of carcasses on consumers has clearly demonstrated the transfer of marine-derived nitrogen and carbon into stream food webs. In particular, isotopic studies have demonstrated importance of carcasses to the carbon and nitrogen content of juvenile fishes (Bilby et al. 1998; Chaloner et al. 2002; Hicks et al. 2005; Kline et al. 1990) and benthic invertebrates (Chaloner et al. 2002; Hicks et al. 2005; Winder et al. 2005). These isotopic studies rely on specific, but untested assumptions regarding fractionation rates of carbon and nitrogen (Gende et al. 2002). The data presented here substantiate those conclusions by demonstrating direct consumption of marine-derived lipids. The reported responses of juvenile salmonids to marine subsidies include increased density (Bilby et al. 1998; Lang et al. 2006) and growth (Giannico and Hinch 2007; Lang et al. 2006; Wipfli et al. 2003; Wipfli et al. 2004). These effects have previously been correlated with concomitant changes in fatty acid composition (Heintz et al. 2004; Volk 2004). The data in this report demonstrate that carcass effects on growth and energy stores result from direct consumption of marine-derived lipids.

Data presented here indicate that direct consumption of marine-derived lipids can have a substantial impact on consumer nutrition. Previously, direct transfer of marine-derived nutrients into consumer tissues has been described as a supplement to bottom-up pathways. However, Hicks et al. (2005) determined carcass tissues accounted for 35% of coho salmon diets in beaver ponds receiving adult salmon based on isotopic mixing

models. In addition, these nutritional benefits likely extend to coho salmon prey (Chaloner et al. 2002; Hicks et al. 2005; Minakawa et al. 2002; Winder et al. 2005) and are likely to have long term effects on the growth of juvenile coho salmon throughout the winter (Giannico and Hinch 2007). Most of the previous demonstrations of increased density (Bilby et al. 1998; Giannico and Hinch 2007; Wipfli et al. 2003; Wipfli et al. 2004) and growth of salmonids (Giannico and Hinch 2007; Wipfli et al. 2003; Wipfli et al. 2004) relied on manipulations of natural and artificial systems. The data for the natural streams in this report demonstrate that direct consumption played an important role in provisioning coho salmon with lipid reserves prior to winter. These provisions were acquired at carcass densities one tenth to one half those used in aforementioned manipulations (Mitchell and Lamberti 2005). The primary benefit observed in conjunction with direct consumption of marine-derived lipids was the provisioning of coho salmon with energy reserves. These fish were sampled at the end of the growing season when day length was rapidly declining. In winter juvenile coho salmon survival can be as low as 15% (Huusko et al. 2007) therefore direct consumption of marine-derived lipid likely influences the production of smolts in spring.

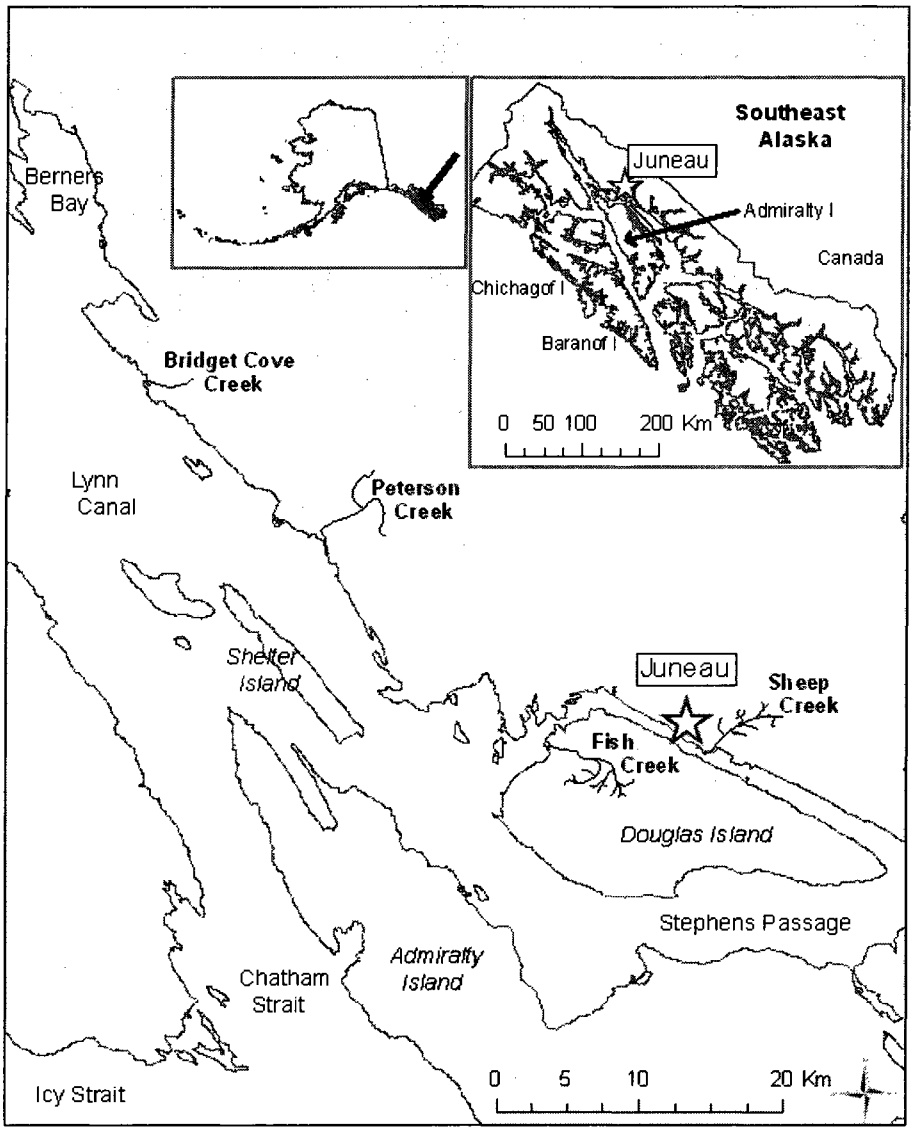

Figure 2.1. Location of mesocosm experiment and natural streams. Natural streams sampled for coho salmon (Bridget Cove Creek, Peterson Creek and Fish Creek). Experiment took place at Sheep Creek.

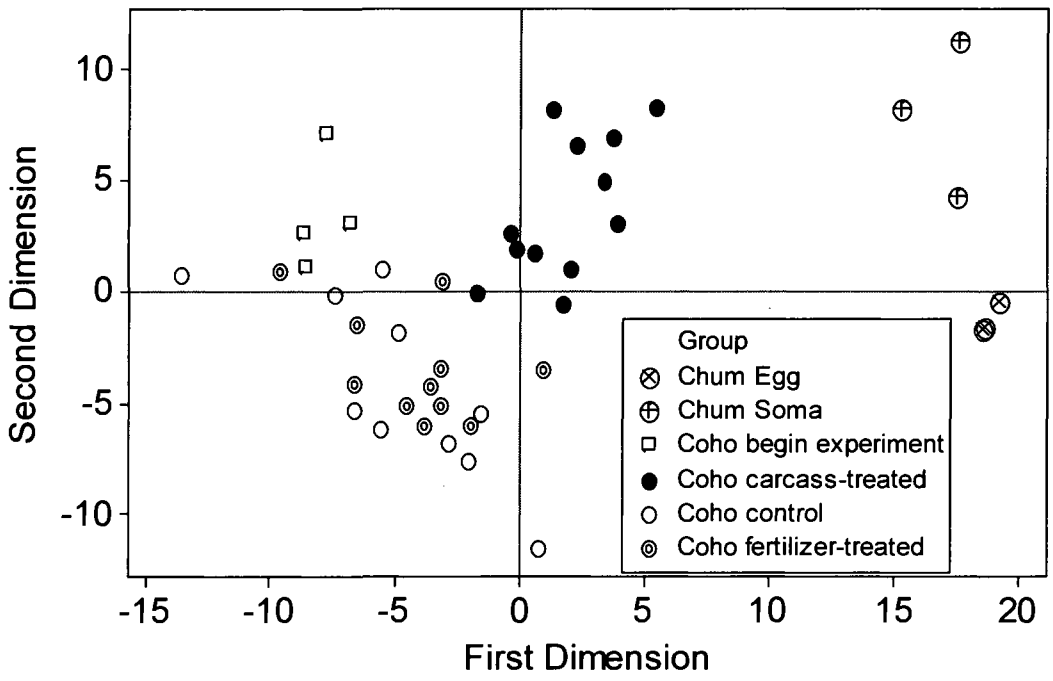

Figure 2.2. NMDS plot of salmon in the mesocosm experiment. Points depict the similarity between the fatty acid compositions of coho and chum salmon (Kruskal's stress = 0.125).

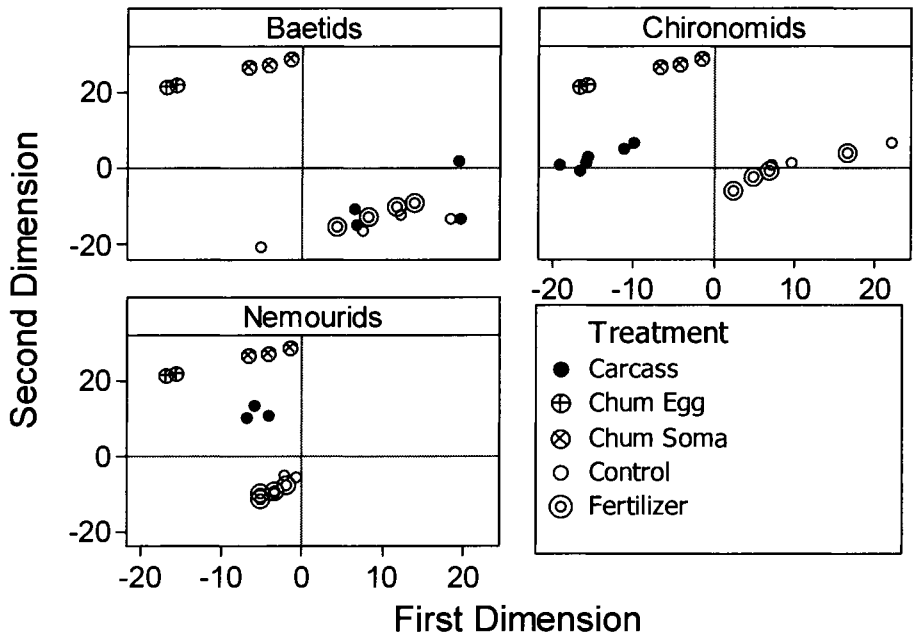

Figure 2.3. NMDS plots of insects in the mesocosm experiment. Points depict similarity between the fatty acid compositions of insect taxa (from carcass, control, and fertilizer treated channels) and chum salmon (eggs and soma). The model was constructed using all insects simultaneously. Taxa are plotted separately to aid in interpretation (Kruskal's stress = 0.143).

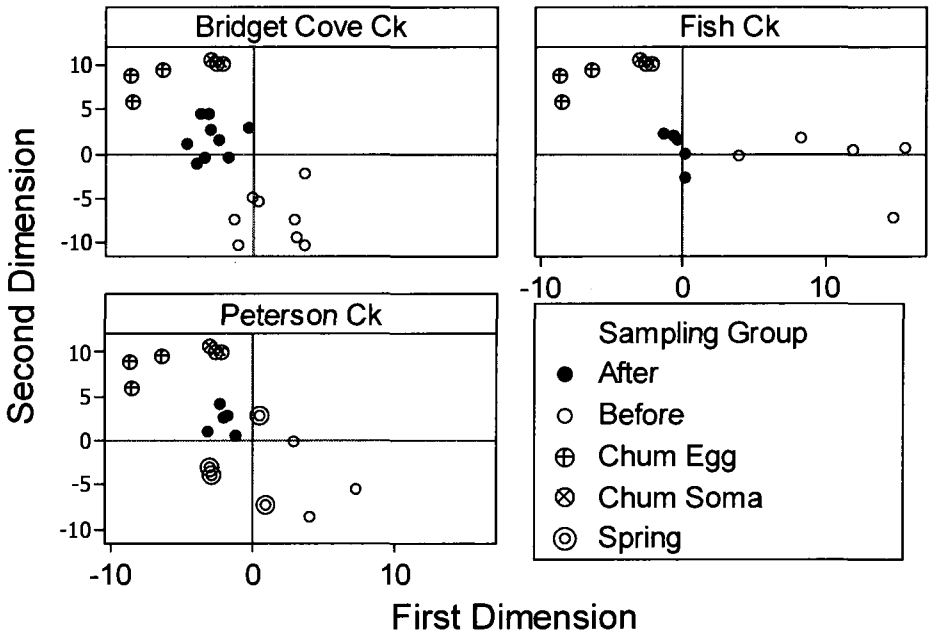

Figure 2.4 NMDS plots of salmon from natural streams. Points depict of juvenile coho and adult chum salmon collected before and after the presence of carcasses. The model was constructed using all fish simultaneously, but specific streams are depicted separately to aid interpretation. Note spring samples depict the fish collected in April from Peterson creek (Kruskal's stress = 0.117).

Table 2.1. Characteristics of coho salmon from mesocosm experiment. Values reflect number, mean (± 1 s.e.) weight (g), lipid (% of wet mass) and lipid composition (% of total lipid). Fish labeled "Initial" are those sampled before the experiment began and representative of the natural energy source. "Control" and "Fertilizer" represent the natural and enhanced fresh water energy sources, respectively. "Carcass' represents the marine-derived energy source. Structural lipids represent the sum of PC, PE and cholesterol.

	Initial	Control	Fertilizer	Carcass
Number sampled	4	12	12	12
Mean weight	1.43 ± 0.11	1.73 ± 0.16	1.70 ± 0.17	2.29 ± 0.19
% Lipid	1.83 ± 0.14	1.78 ± 0.13	2.10 ± 0.23	3.43 ± 0.27
% TAG	21.7 ± 3.5	18.9 ± 4.3	21.1 ± 5.4	47.4 ± 3.8
% Structural lipid	29.7 ± 1.0	47.8 ± 3.9	41.4 ± 4.1	23.2 ± 1.7

Table 2.2. Characteristics of insects from mesocosm experiment. Values show number and mean (± 1 s.e.) individual weight (mg), lipid (% of wet mass) and lipid composition (% of total lipid). Insects from a given channel were composited into a single taxon-specific sample to ensure sufficient sample mass. The number of insects in a given composite varied. Channel treatments are the same as those described in table 2.1.

	Control	Fertilizer	Carcass
Baetids			
Number weighed	17	21	26
Mean weight	2.47 ± 0.08	3.22 ± 0.08	3.05 ± 0.19
Number composites	4	4	3
% Lipid	8.17 ± 2.03	6.05 ± 0.59	6.69 ± 1.63
Nemourids			
Number sampled	24	24	18
Mean weight	1.43 ± 0.04	1.73 ± 0.10	1.99 ± 0.12
Number composites	4	4	3
% Lipid	3.21 ± 0.37	3.37 ± 0.23	4.86 ± 0.96

Table 2.2, continued.

	Control	Fertilizer	Carcass
Chironomids			
Number sampled	23	24	25
Mean weight	2.11 ± 0.10	3.30 ± 0.21	4.80 ± 0.24
Number composites	4	4	4
% Lipid	2.91 ± 0.70	2.75 ± 0.37	4.56 ± 0.20
% TAG	11.17 ± 1.2	16.05 ± 1.5	51.90 ± 2.5
% Structural lipids	60.1 ± 0.8	50.5 ± 4.0	29.9 ± 1.8

Table 2.3. Lipid composition of coho salmon in natural streams. Values show mean (± 1 s.e.) individual weight (g), lipid (% of wet mass) and lipid composition (% of total lipid) before and after the arrival of adult salmon. Only Peterson Ck was sampled in spring 2001. Structural lipids represent the sum of PC, PE and cholesterol.

	Spring	Before	After
Peterson Ck			
Number sampled	5	6	5
Sample date	18 Apr.	2 Jul.	2 Oct.
Mean weight	3.70 ± 0.42	0.90 ± 0.04	2.45 ± 0.41
% Lipid	2.12 ± 0.22	1.44 ± 0.19	4.12 ± 0.41
% TAG	26.50 ± 6.6	14.21 ± 7.2	53.3 ± 3.1
% Structure	44.5 ± 7.0	52.5 ± 5.1	21.8 ± 2.6
Bridget Cove Ck			
Number sampled		11	9
Sample date		9-12 Jul.	20 Sep.
Mean weight		1.48 ± 0.35	2.65 ± 0.14
% Lipid		1.69 ± 0.16	5.16 ± 0.53
% TAG		19.8 ± 6.2	44.9 ± 3.6
% Structural lipids		51.5 ± 5.7	24.5 ± 1.8

Table 2.3, continued.

	Spring	Before	After
Fish Ck			
Number sampled		8	6
Sample date		29 Jun.	29 Sep.
Mean weight		0.44 ± 0.03	1.82 ± 0.29
% Lipid		1.59 ± 0.14	2.84 ± 0.30
% TAG		9.1 ± 2.3	35.3 ± 6.9
% Structural lipids		59.8 ± 1.8	34.9 ± 3.1

Table 2.4. Fatty acid compositions of insects from control channels. Collections made at the end of the mesocosm experiment. Values are mean (± 1 s.e.) % of total fatty acids observed. "n.d." indicates the fatty acid was not detected. "*" indicates detection in only 1 sample. "c" indicates cis, t indicates trans. Co-eluting peaks are identified as a sum.

	Baetids	Chironomids	Nemourids
14:0	4.93 ± 0.94	11.95 ± 4.17	5.57 ± 2.50
14:1n5	0.04 ± 0.01	0.13 ± 0.04	0.02 ± 0.00
15:0	0.39 ± 0.02	0.87 ± 0.24	1.02 ± 0.43
15:1n5	0.01 *	0.01*	0.01*
16:0	30.69 ± 4.18	26.44 ± 8.15	23.29 ± 10.60
16:1n7	9.60 ± 0.38	6.22 ± 2.16	7.73 ± 0.13
17:0	0.70 ± 0.05	1.40 ± 0.40	2.15 ± 0.87
17:1n7	n.d.	0.12 *	0.13*
18:0	4.94 ± 0.43	8.87 ± 3.04	9.55 ± 4.01
18:1n9 c&t	6.84 ± 0.63	7.77 ± 2.59	20.01 ± 0.19
18:1n7	8.06 ± 0.81	2.55 ± 0.93	1.82 ± 0.19
18:2n6 c	3.35 ± 0.48	7.69 ± 2.63	13.82 ± 0.56
18:2n6 t	n.d.	0.20*	n.d.
18:3n6	0.28 ± 0.04	0.45 ± 0.04	0.86 ± 0.10
18:3n3	14.21 ± 2.11	8.32 ± 2.91	15.85 ± 0.44
20:0	0.12 ± 0.01	1.84 ± 0.67	0.44 ± 0.18
20:1n9+11	0.07 ± 0.01	0.39 ± 0.10	0.19 ± 0.04
20:2n6	0.04 ± 0.00	0.06 ± 0.01	0.10 ± 0.01
20:3n6	0.03 ± 0.01	0.05 ± 0.00	0.09 ± 0.01
20:4n6	1.01 ± 0.28	1.17 ± 0.47	2.75 ± 0.07

Table 2.4, continued.

	Baetids	Chironomids	Nemourids
20:3n3	0.22 ± 0.05	0.06 ± 0.03	0.15 ± 0.04
20:5n3	12.66 ± 1.81	11.05 ± 4.03	11.57 ± 0.55
22:0	0.12 ± 0.01	0.12 ± 0.03	0.22 ± 0.08
22:1n9+11	1.37 ± 0.73	2.29 ± 0.81	0.79 ± 0.21
22:2n6	0.03 ± 0.01	0.05 ± 0.01	0.02 ± 0.00
22:5n3	0.03 ± 0.01	0.09 ± 0.02	0.11 ± 0.02
22:6n3	0.22 ± 0.03	0.23 ± 0.10	0.43 ± 0.14
24:0	0.05 ± 0.01	0.07 ± 0.02	0.10 ± 0.02
24:1n9	0.03 ± 0.01	0.06 ± 0.00	0.03 ± 0.00
Sum Sat'd	41.94 ± 5.52	51.56 ± 16.63	42.34 ± 18.69
Sum Mono	26.00 ± 1.08	19.37 ± 6.45	23.23 ± 7.24
Sum n3	27.34 ± 3.82	19.72 ± 7.03	21.20 ± 7.07
Sum n6	4.72 ± 0.81	9.36 ± 3.26	17.64 ± 0.51
n3/n6	5.90 ± 0.28	2.38 ± 0.32	1.61 ± 0.08

Table 2.5. Fatty acid compositions of initial coho and chum salmon tissues used in the mesocosm experiment. Values are mean (± 1 s.e.) % of total fatty acids observed. Coho and chum salmon samples were collected at the beginning of the experiment. Abbreviations are as in Table 2.4.

	Initial coho	Chum ova	Chum soma
14:0	5.90 ± 2.33	4.27 ± 0.23	3.42 ± 0.17
14:1n5	0.12 ± 0.05	0.14 ± 0.01	0.03 ± 0.01
15:0	0.38 ± 0.08	1.07 ± 0.08	0.60 ± 0.01
15:1n5	n.d.	0.02 *	n.d.
16:0	14.53 ± 0.65	8.68 ± 0.99	13.81 ± 0.86
16:1n7	8.51 ± 0.83	6.57 ± 0.24	3.91 ± 0.16
17:0	0.60 ± 0.10	0.75 ± 0.06	0.50 ± 0.04
17:1n7	n.d.	n.d.	n.d.
18:0	6.38 ± 0.62	6.98 ± 0.43	4.50 ± 0.14
18:1n9 c&t	27.92 ± 0.83	20.47 ± 0.59	15.65 ± 2.12
18:1n7	3.02 ± 0.65	3.22 ± 0.22	3.29 ± 0.33
18:2n6 c	12.16 ± 0.39	2.02 ± 0.10	1.70 ± 0.03
18:2n6 t	n.d.	n.d.	n.d.
18:3n6	1.24 ± 0.14	0.08 ± 0.01	0.09 ± 0.03
18:3n3	9.27 ± 1.08	1.69 ± 0.08	1.36 ± 0.16
20:0	0.63 ± 0.05	0.08 ± 0.00	0.17 ± 0.04
20:1n9+11	0.95 ± 0.14	2.10 ± 0.12	5.58 ± 1.06
20:2n6	0.48 ± 0.10	0.67 ± 0.04	0.51 ± 0.06
20:3n6	0.42 ± 0.07	0.28 ± 0.01	0.17 ± 0.05
20:4n6	1.39 ± 0.30	2.56 ± 0.12	1.84 ± 0.09

Table 2.5, continued.

	Initial coho	Chum ova	Chum soma
20:3n3	0.24 ± 0.04	0.47 ± 0.02	0.13 ± 0.05
20:5n3	1.96 ± 0.21	14.25 ± 0.31	9.53 ± 0.47
22:0	0.15 ± 0.01	n.d.	0.03 ± 0.01
22:1n9+11	0.16 ± 0.02	0.69 ± 0.04	5.86 ± 0.83
22:2n6	0.09 ± 0.01	0.02 ± 0.00	0.04 ± 0.01
22:5n3	0.83 ± 0.13	7.49 ± 0.32	3.97 ± 0.34
22:6n3	2.62 ± 0.30	17.55 ± 0.64	22.65 ± 2.72
24:0	0.04*	n.d.	0.03*
24:1n9	0.08 ± 0.01	0.11 ± 0.01	0.63 ± 0.21
Sum Sat'd	28.58 ± 1.79	21.83 ± 0.28	23.05 ± 0.67
Sum Mono	40.76 ± 0.74	33.30 ± 0.69	34.95 ± 3.47
Sum n3	14.91 ± 1.06	41.45 ± 1.36	37.64 ± 3.20
Sum n6	15.75 ± 0.88	5.61 ± 0.27	4.35 ± 0.13
n3/n6	0.95 ± 0.03	7.40 ± 0.17	8.71 ± 0.97

Table 2.6. Fatty acid compositions of coho salmon in mesocosm experiment. Numbers are mean (± 1 s.e.) percent of total fatty acids at the end of the mesocosm experiment. Abbreviations are as in Table 2.4.

	Control	Carcass	Fertilizer
N	12	12	12
14:0	4.14 ± 0.33	3.57 ± 0.10	4.42 ± 0.21
14:1n5	0.06 ± 0.01	0.07 ± 0.01	0.13 ± 0.03
15:0	0.37 ± 0.06	0.47 ± 0.06	0.42 ± 0.08
15:1n5	0.12*	0.04 ± 0.02	0.10*
16:0	16.85 ± 0.55	18.16 ± 0.70	17.50 ± 0.45
16:1n7	8.03 ± 0.37	11.64 ± 0.68	9.93 ± 0.57
17:0	0.79 ± 0.06	0.56 ± 0.06	0.72 ± 0.07
17:1n7	n.d.	n.d.	n.d.
18:0	8.58 ± 1.04	5.84 ± 0.40	6.66 ± 0.21
18:1n9 c&t	16.40 ± 0.80	19.20 ± 0.65	17.74 ± 0.78
18:1n7	5.46 ± 0.37	6.84 ± 0.34	5.65 ± 0.15
18:2n6c	11.88 ± 0.73	6.83 ± 1.01	10.39 ± 0.71
18:2n6t	n.d.	n.d.	n.d.
18:3n6	0.97 ± 0.08	0.69 ± 0.08	0.87 ± 0.06
18:3n3	13.74 ± 0.74	7.70 ± 0.47	12.59 ± 1.01
20:0	0.81 ± 0.22	0.44 ± 0.08	0.50 ± 0.03
20:1n9+11	0.66 ± 0.10	2.35 ± 0.44	0.87 ± 0.34
20:2n6	0.27 ± 0.08	0.29 ± 0.03	0.26 ± 0.03
20:3n6	0.30 ± 0.05	0.26 ± 0.03	0.28 ± 0.02
20:4n6	1.42 ± 0.13	0.93 ± 0.08	1.19 ± 0.09

Table 2.6, continued.

	Control	Carcass	Fertilizer
20:3n3	0.27 ± 0.03	0.25 ± 0.01	0.26 ± 0.01
20:5n3	3.97 ± 0.30	3.33 ± 0.18	3.88 ± 0.30
22:0	0.20 ± 0.04	0.12 ± 0.02	0.14 ± 0.02
22:1n9+11	0.12 ± 0.02	1.40 ± 0.34	0.46 ± 0.37
22:2n6	0.05 ± 0.01	0.04 ± 0.00	0.06 ± 0.00
22:5n3	1.14 ± 0.09	1.29 ± 0.12	1.17 ± 0.13
22:6n3	3.65 ± 0.29	7.40 ± 0.95	4.08 ± 0.48
24:0	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
24:1n9	0.06 ± 0.01	0.33 ± 0.07	0.12 ± 0.06
Sum Sat	31.71 ± 0.88	29.16 ± 0.77	30.31 ± 0.55
Sum Mono	30.74 ± 1.02	41.83 ± 0.82	34.75 ± 1.61
Sum n3	22.73 ± 1.16	19.98 ± 0.70	21.98 ± 1.06
Sum n6	14.82 ± 0.80	9.03 ± 1.19	12.97 ± 0.74
n3/n6	1.61 ± 0.15	2.57 ± 0.28	1.74 ± 0.10

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Appendices

Appendix 2.1. ANOVA results for coho salmon in mesocosm experiment. Treatment was a fixed factor and table was random. See equation 2.1 for model.

Response	Source	DF	Mean square	F
Weight	Treatment	2	1.306	30.67
	Table	5	0.149	3.5
	Treatment×Table	10	0.043	0.07
	Error	18	0.614	
% Lipid	Treatment	2	9.178	23.59
	Table	5	0.221	0.57
	Treatment×Table	10	0.389	0.51
	Error	18	0.760	
%TAG	Treatment	2	0.302	47.59
	Table	5	0.006	1.01
	Treatment×Table	10	0.006	0.16
	Error	18	0.040	
%Structure	Treatment	2	0.195	50.27
	Table	5	0.004	1.03
	Treatment×Table	10	0.004	0.17
	Error	18	0.023	

Appendix 2.2. ANOVA results for insects in mesocosm experiment. Treatment was a fixed factor and table was random. Model was the same as show in equation 2.1 except it was not possible to test the interaction term.

Response	Source	DF	Mean square	F
	Baetids			
Weight	Treatment	2	0.589	1.34
	Table	4	0.144	0.33
	Error	6	0.439	
% Lipid	Treatment	2	4.54	0.39
	Table	3	3.87	0.34
	Error	5	11.56	
	Nemourids			
Weight	Treatment	2	0.125	0.58
	Table	5	0.567	2.63
	Error	18	0.216	
% Lipid	Treatment	2	2.350	4.21
	Table	3	1.655	2.97
	Error	5	0.558	

Appendix 2.2 continued.

Chironomids				
Response	Source	DF	Mean square	F
Weight	Treatment	2	7.311	11.03
	Table	3	1.325	2.00
	Error	6	0.663	
% Lipid	Treatment	2	3.992	3.11
	Table	3	0.089	0.974
	Error	6	1.285	
%TAG	Treatment	2	0.140	23.72
	Table	3	0.005	0.90
	Error	3	0.006	
%Structure	Treatment	2	0.053	70.06
	Table	3	0.0004	0.65
	Error	3	0.0007	

Appendix 2.3. ANOVA results for coho salmon in natural streams. Fish sampled before and after the arrival of adult salmon. In addition fish were sampled in spring from one of the streams. Model for the ANOVAs is given in equation 2.2 where sampling period is fixed and location is a random factor nested within period.

Response	Source	DF	Mean square	F
% Lipid	Sampling period	2	32.468	43.38
	Location (period)	4	4.915	6.57
	Error	43	0.478	
% TAG	Sampling period	2	0.476	21.55
	Location (period)	4	0.036	1.62
	Error	43	0.022	
% Structure	Sampling period	2	0.398	27.72
	Location (period)	4	0.023	1.60
	Error	43	0.014	

Chapter III

Effects of Diet Quality on Energy Loss in Fasting Coho Salmon (*Oncorhynchus kisutch*)[4]

Abstract

Juvenile coho salmon are known to consume marine-derived tissues when adult salmon return to spawn in their natal streams. These tissues, such as salmon eggs, are highly digestible and can be abundant in streams. However, it is not known how consumption of these tissues influences the potential survival of coho salmon over winter. This study examined how diets of varying quality influenced the body composition, metabolic rate and the substrates used to meet metabolic demand in juvenile coho salmon fasted under controlled conditions. The data are compared to the performance of wild coho salmon collected during two winters. Results of the laboratory studies indicate that a diet of pink salmon tissue provided juvenile coho salmon with higher lipid levels and metabolic rates than a chironomid diet. In addition, temperature had far reaching effects on the relationships between dietary lipid to protein ratios, metabolic rate and energy allocation strategies. At low temperatures (4.0 °C) during feeding fish were able to allocate greater proportions of mass to lipid than those fed at higher temperatures (5.9 °C). The converse was true during fasting; fish at higher

[4] Heintz, R.A. Effect of diet quality on energy loss in fasting coho salmon (*Oncorhynchus kisutch*). Prepared for submission in Transactions of the American Fisheries Society.

temperatures (3.0 °C) lost proportionately more lipid than those at lower temperatures (0.3 °C). Wild fish, experienced significant declines in lipid but maintained dry mass during both winters indicating that they actively fed during winter. These data indicate that consumption of adult salmon tissues provisions juvenile salmonids with lipid that can be used to maintain high metabolic rates and while forestalling mass and energy loss during winter. These effects will be most beneficial if coho experience low temperatures during feeding and fasting.

Introduction

Juvenile coho salmon (*Oncorhynchus kisutch*) gain increased access to energy when adult salmon return to spawn in their natal streams. Redd excavation by adult salmon dislodges benthic macro-invertebrates that can be consumed by juvenile fish located downstream from spawning habitat (Minakawa and Gara 2003). In addition, juvenile salmon can also consume eggs and other adult salmon tissues (Eastman 1996; Koski and Kirchhofer 1982; Scheurell et al. 2007). This increase in the availability of food can be an important source of nutrition. Juvenile rainbow trout (*O. mykiss*) and Arctic char (*Thymallus arcticus*) increased their daily consumption rates by 500% following the arrival of adult sockeye salmon (Scheurell et al. 2007). Increased consumption rates are consistent with the observation that most of the particulate organic carbon in British Columbian streams came from carcass tissues after adult salmon arrived (Johnston et al. 2004). Increased access to energy translates to increased growth (Lang et al. 2006; Wipfli et al. 2003; Wipfli et al. 2004) and lipid levels (Heintz et al. 2004; Wipfli et al. 2004) for juvenile salmonids.

The potential benefits afforded juvenile salmonids may also be accentuated by increases in the quality of the food offered by adult salmon carcass tissues. Aquaculture research has identified the components of salmon diets that maximize growth. These components include essential amino acids and fatty acids that cannot be synthesized *de novo*, low chitin content and sufficient lipid to allow protein to be spared for tissue growth (Higgs et al. 1995). These are all features of adult salmon tissues and eggs. Ogata et al. (1983) found increased growth among juvenile salmon fed diets with amino acid

compositions consistent with that of juvenile's whole body protein. Lipid to protein ratios in Pacific salmon eggs (0.39) (unpublished data) are approximately equal to the ratio recommended for coho salmon (Higgs et al. 1995). The absence of chitin in salmon tissues suggests a higher digestibility for adult salmon tissues and therefore improved net energy gains over consumption of benthic macro-invertebrates.

The increased lipid levels observed among juvenile salmon that consume marine tissues (Heintz et al. 2004; Lang et al. 2006; Wipfli et al. 2004) are likely to increase their potential survival over winter. Estimates of overwinter mortality range as high as 84% for coho salmon (Huusko et al. 2007). Mortality of juvenile salmon during winter is thought to relate directly to mismatches between energy intake and demand (Hurst 2007). For example, in early winter cold water temperatures reduce appetite (Metcalfe and Thorpe 1992), but at the same time, acclimation to falling water temperatures is energetically demanding (Cunjak 1988). In addition, rapid increases in stream discharge associated with thawing can impose further energetic demands. Increased lipid reserves at winter's onset may buffer fish from these demands, but once these reserves become depleted fish must forage (Bull et al. 1996). However, several reports indicate that winter foraging may not return sufficient energy to maintain growth and survival (Giannico and Hinch 2003; Quinn and Peterson 1996). In addition, increased foraging places juvenile salmonids at greater risk of predation.

The problems faced by juvenile salmonids in winter are exacerbated for small fish. The allometries between metabolic demand and size and energy storage and size determine the amount of energy lost by fish over winter (Schultz and Conover 1999).

Larger fish have lower mass-specific metabolic rates than smaller fish and can therefore store more energy conversely small fish have high metabolic rates and reduced capacity for energy storage. These discrepancies are thought to account for observations of size-selective mortality in winter for coho salmon (Giannico and Hinch 2007; Quinn and Peterson 1996). Thus, improved growth and increased lipid levels observed among juvenile salmonids following the arrival of adult salmon suggests that adult salmon tissues may play in important role in determining winter survival of juveniles.

The goals of this study were to determine the potential benefits gained by wild coho salmon eating salmon carcass tissues prior to a winter fast and relate those benefits to changes in the nutritional status of these fish during winter. In particular, I examine the effect of diet on body composition and the effect of diet on metabolic rate and on the substrates used to meet metabolic demand during a fast. Experiments aimed at these objectives were performed over two winters. The results of those experiments were compared to changes in body composition and energy content of wild coho salmon sampled from a natural stream during those same winters.

Methods

Experiments were conducted over the winters of 2004-2005 and 2005-2006 to test the hypothesis that foraging on high quality foods has long term effects on survival when food supplies become scarce. In the first of these experiments, hereafter referred to as Year 1, fish were fed chironomids, ground beef or pink salmon (*Oncorhynchus gorbuscha*) for 91 days and then starved for 59 days. The diets offered in Year 1 represent a range of lipid to protein ratios known to sustain coho salmon. In the second

experiment, referred to as Year 2, fish were fed chironomids or diets formulated with either beef tallow or salmon oil for 70 days and then sampled periodically as they fasted for 43 days. The formulated diets offered in Year 2 were similar to each other, but differed in fatty acid composition. In turn, they differed from the chironomids in lipid to protein ratio.

Year 1 experiment

The objective of the experiment performed in Year 1 was to compare the effects of eating different diets on the body composition and fasting performance. Chironomids are natural prey for coho salmon but have a low lipid to protein ratio and contain chitin, suggesting they are of lower quality than salmon tissue. Beef has no chitin but an extremely high lipid to protein ratio, suggesting that it may also be inferior to salmon tissue. Wild coho salmon were collected from Peterson Creek, near Juneau AK (58° 37' 16" N, 134° 56' 11" W) on September 23, 2004 and transported to a laboratory. Fish were reared and fed a commercial fish diet *ad libitum* for 47 days to normalize their body compositions. On November 6, 2004, the length of each fish was recorded and the fish were randomly assigned to one of six tanks; 17 fish in each of four tanks and 9 fish in each of two smaller tanks. Density of fish was the same in all tanks; the larger tanks held 50 L of water and the smaller tanks held 25 L. Fish in the tanks holding 17 fish received either "high" or "low" rations of ground beef or pink salmon. Fish in the tanks holding 9 fish received a diet consisting of commercially purchased chironomids. The tanks were supplied with ambient fresh water from a nearby lake and temperatures averaged 4.0° C while feeding the different diets. Day lengths of approximately 13 hours were maintained

using artificial lighting. The beef and chironomids were purchased locally and kept frozen during the feeding period. The adult pink salmon was obtained from a nearby salmon stream, ground in a commercial meat grinder and frozen.

Fish were fed different weights of food so that rations offered high-ration fish were isocaloric while rations offered low-ration and chironomid-fed fish were roughly half those of the high-ration fish. Despite the differences in ration, it appeared all fish were likely consuming equal amounts because there was excess food on the bottom of all the tanks. Energy content of the food was determined from its proximate composition and associated calorific equivalents, 36.43 kJ/g lipid, 20.10 kJ/g protein and 17.11 kJ/g carbohydrate (Brett 1995). Feeding continued daily for 91 days.

Fasting began on February 8, 2005. Each of the fish were removed from their tanks, anaesthetized and measured for fork length and wet weight. A random set of 5 fish was collected from each of the diet groups, to determine their initial condition by proximate analysis. The remaining 12 fish were placed into individually marked 25 mm diameter vinyl tubes cut to approximately 150% of the fish's length. Wire screening was fixed onto either end of the tubes and the tubes were placed in a tank with flowing ambient lake water (average temperature 3.25° C). The tubes restricted fish movement so that energy expenditures represented those of resting fish in a post-absorptive state. The fish were placed facing upstream, but flows were sufficiently low that fish could maintain position without actively swimming. Periodic inspection revealed that most of the time fish rested against the side of the tubes with their fins folded. The tank was covered and

the fish remained there for 59 days. On April 5, 2005 the fish were removed from the tubes, to record their lengths, weights and proximate compositions.

Year 2 experiment

The objective of the Year 2 experiment was to repeat the Year 1 experiment, but reduce the extent to which diet quality differed. The ground beef and fish diets were replaced with formulated diets in which the fat was derived from salmon oil or beef tallow. Chironomids were offered to a third group to provide comparability with Year 1. Procedurally the approach was similar to that of Year 1, but failure of the lab's water supply forced adoption of a static culture system. Fish were collected from Montana Creek (58° 22' 54" N, 134° 35' 43" W) near Juneau, Alaska on November 26, 2005 and transported to a wet lab where 30 fish were randomly assigned to each of three 80 L tanks. The tanks were bathed in running seawater to maintain low temperatures (average 5.9° C). Air was supplied to each tank to maintain adequate oxygen levels and the water in the tanks was exchanged once a week during the feeding period.

Fish fasted for a week and then introduced to the experimental diets: chironomids, beef-fat and fish-fat. The beef-fat and fish-fat diets were produced in the lab (Table 3.1). Initially, the beef-fat and fish-fat diets were isonitrogenous and isocaloric differing only in lipid source. The beef-fat diet used beef tallow and primrose oil as lipid sources while the fish-fat diet used salmon oil. Fish readily ate the chironomids and beef-fat diet, but were reluctant to eat the fish-fat diet. To make the fish-fat diet more palatable it was amended with 22% ground pink salmon, altering its protein and lipid content (Table 3.2).

Fish were fed every other day for three weeks until they began aggressively feeding at which point they were fed daily. Feeding continued for 60 days.

On February 6, 2006, feeding ceased and fish were transferred back to Montana Creek for fasting. The lengths and weights of all the fish were recorded and a sample of 8 to 9 fish from each diet was retained for proximate analysis. The remaining fish were placed in vinyl tubes as in Year 1. The tubes were placed inside minnow traps and suspended in a small side channel of Montana Creek. Visual inspection indicated that the fish faced upstream and could maintain their position in the tubes without actively swimming. The tubes were placed in 3 minnow traps so that samples could be collected on days 16, (February 22), 29 (March 7) and 43 (March 21) of the fast without disturbing the other fish. In addition, 29 wild coho salmon were also collected. These fish were obtained by trapping fish in Montana Creek during the same period in which the feeding was ending. They are hereafter referred to as the "Captive-wild" group, their diets were considered "natural". On February 6, a sample of 9 of these fish was retained for proximate analysis and an additional 20 were placed in vinyl tubes and put into minnow traps with the other fish. On each of the sample dates, a minnow trap was removed. The fish were measured for length and weight, killed with a blow to the head and immediately frozen for later analysis. The study plan called for sampling five fish from each group on days 16 and 29 and 10 fish from each group on day 43. However, sample sizes varied during the fast due to escapes.

Energy loss over winter in wild coho salmon

Sampling fish from Montana Creek while both the feeding experiments were underway provided a context for understanding the energetics of wild fish in winter. Temperature was recorded at fixed stations in both years. Discharge measurements during the sampling period were obtained from the United States Geological Survey who maintains a gauging station approximately 500m downstream from the study area. Temperature was recorded by deploying a recording thermometer in the mouth of a side channel during the sampling period. Fish were collected from the stream in both years using baited minnow traps. Sample sites depended on where fish could be caught, but generally they were located within a 200m diameter range of the site where fish were fasted in Year 2. Locations included logjams in the main stem and the mouths of side channels. Sample collections were timed to coincide with the onset of feeding, end of feeding and at multiple times during each fast. The lengths and weights of collected fish were recorded and they were stored for proximate analysis.

Proximate Analysis

All fish collected for proximate analysis were stored at -80°C between sampling and analysis. The mass-specific energy content (dry mass basis) of fish and diets in Year 1 were determined from the calorific equivalents for lipid, protein and carbohydrate (Brett 1995): 36.43 kJ/g lipid, 20.10 kJ/g protein and 17.11 kJ/g carbohydrate. Small sample sizes often prevented analysis of the ash content of the fish. The energy content of fish sampled in Year 2 was determined by bomb calorimetry. Protein and ash were only estimated for a small number of individuals in Year 2. However, 32 of the samples in Year

2 were large enough to provide for complete proximate analysis and bomb calorimetry. These samples were used to verify that energy content estimates from calorific equivalents were the same as those derived from calorimetric estimates. Stomachs were removed from all individuals prior to analysis. Those taken from fish in Year 2 were inspected to determine what proportion contained identifiable remains. Initially all fish were cut into small pieces and then homogenized with a mortar and pestle. An aliquot (0.3 g) of each sample was removed for lipid analysis, placed in vial and topped with nitrogen. The remaining sample was dried, and analyzed for protein or energy content. In the case where both the nitrogen and calorimetric analyses were performed, the wet tissue was divided into two aliquots. One was dried and analyzed in the calorimeter; the other was dried and analyzed for nitrogen.

Lipid was extracted from 0.5 to 1.0 g of wet sample homogenate using a modification of Folch's method outlined by Christie (1982) in a Dionex Accelerated Solvent Extractor (ASE) 200 with 2:1 (v:v) chloroform:methanol. Extracts were washed successively with a 0.88% KCl solution and 1:1 (v:v) methanol:deionized water in a volume equal to 25% of the extract volume to remove co-extractables. Excess solvent was evaporated and percent lipid was calculated gravimetrically. Quality assurance samples included with each batch of 17 lipid samples consisted of a blank to control for cross contamination, a reference material to determine accuracy and a duplicated sample to evaluate repeatability. References were all found to be within 10% of the expected value, and duplicated samples were always within 5% of each other.

Moisture and ash content were determined with a Leco TGA-601 thermogravimetric analyzer. Approximately 2.5 g of wet homogenate were placed in a crucible inside the instrument. The temperature was increased from ambient to 135 °C over 6 minutes and then held until repeated measurements of the mass varied by less than 5%. The temperature was subsequently increased to 600 °C and held until repeated measurements of mass varied by less than 5%. Moisture content was estimated as the difference in initial mass and the stable mass at 135 °C. Ash content was estimated as the stabilized mass observed at 600 °C. Quality assurance for moisture and ash analyses included blank and duplicated samples. If the initial sample mass was less than 2.5 g, ash weight was not determined.

Protein content was estimated from the total nitrogen content observed in a 0.1 g sample of dried homogenate. Nitrogen content of the sample was measured with a LECO FP 528 Nitrogen Analyzer following the Dumas method in which the homogenate was combusted at 850 °C and the expelled nitrogen measured by thermal conductivity. Total mass of protein was estimated by multiplying the total nitrogen content by 6.25 (Jones 1931, Craig et al. 1978). The instrument was calibrated daily using EDTA. Quality assurance samples included with each batch of 17 samples included a blank reference consisting of pure cane sugar, and a standard reference material (SRM1546) obtained from the National Institute of Standards and Technology (NIST). In addition, all analyses were duplicated to ensure the coefficients of variation for estimated nitrogen content was less than 15%. Final quality control involved summing the estimated percentages of nitrogen, lipid, ash and water and ensuring that the sum fell within 1.5% of 100%.

Calorimetric analysis was performed on dried samples, using a Parr 1425 semi-micro bomb calorimeter. Benzoic acid was used as a standard. Sample masses were designed to have at least five times more energy than the standard deviation of the estimate for the standard.

Data Analysis: Effect of diet on body composition

The effect of diet on fish body composition was examined by linear regression of each fish's lipid to protein ratio on the lipid to protein ratios of their respective diets. In order to account for differences between years, the data were analyzed by an ANCOVA where the relationships between dietary and consumer lipid to protein ratios were blocked on different years. Blocking accounted for differences in temperature between years. In addition, the mean percent lipid and protein content of fish after feeding in Year 1 was examined using a nested ANOVA with diet as the main factor and ration as the nested factor. Ration was considered a nested factor owing to the fact that there was only one ration level for the chironomid-fed fish, but they were held in two tanks. Differences between pairs of diets were contrasted using Tukeys post-hoc pairwise comparisons. All response variables are expressed on a dry mass basis. Fish from Year 2 were examined using a one-way design with diet group as the fixed main factor.

Data Analysis: Effect of diet on metabolic rate

The effect of diet on metabolic rate during the fast was examined by ANOVA. In Year 1 the ANOVA was a one-way model with diet as a fixed factor. In Year 2, an

ANCOVA was performed using diet as the main factor and time as a covariate. Metabolic rate (MR) (kj $\cdot g^{-1} \cdot d^{-1}$) for a given individual as estimated by:

$$MR = \frac{\Delta E}{\bar{w}} \times t^{-1} \qquad \text{(Equation 3.1)}$$

where ΔE is the change in total energy content during the fast, t is the duration of the fast in days and $\bar{w}$ is the geometric mean wet weight of the fish during the fast. Values of total energy were estimated by converting the total lipid and protein content of fish into energy using calorific equivalents. In order to estimate ΔE it was necessary to estimate the initial energy content of the fish. The initial energy content (E_i) was estimated by:

$$E_i = \frac{E_f}{(p)} \qquad \text{(Equation 3.2)}$$

where E_f is the observed total energy content at the end of the fast and p is the proportional change in energy for the starved fish.

The proportional change (p) was calculated from linear regressions relating total energy (E) and length for fish at the beginning and end of the fast. The regressions were compared using ANCOVA with a common slope and p was found by dividing the intercept for fish at the beginning of the fast into the intercept for fish at the end. The ANCOVA model was:

$$E = \text{Length} + \text{Diet} + \text{Time (Diet)} \qquad \text{(Equation 3.3)}$$

Diet was considered a fixed factor and time was nested in diet. In Year 1 there were three levels of diet and two levels of time: before and after. In Year 2, there were 4 levels of diet, the laboratory fed diets and the natural diet embodied in the Captive-wild fish. In addition, there were 4 levels of time: days 0, 16, 29 and 43. There were too few individuals sampled on a given day to adequately model the loss in energy between periods for each of the diets. Consequently, I used a one-way ANCOVA to estimate the loss in energy across all diets on each of the successive dates.

$$E = \text{Length} + \text{Day} \qquad \text{(Equation 3.4)}$$

Under this model I assumed that the proportional loss in energy was the same across diets and calculated p by averaging the loss across diets for days 16, 29 and 43 relative to day 0. Lengths and total energy were converted to their logarithms prior to the analysis. All of the appropriate two and three-way interactions were examined to verify that the slopes relating energy and length were parallel across all sampling strata.

Data Analysis: Substrates used to meet metabolic demand

The relationship between body composition and the substrates used to meet metabolic demand was examined by ANCOVA. The average contribution of lipid to metabolic demand during was regressed on the lipid to protein ratio for each of the diets blocked on both years. The blocking accounted for temperature differences between years. The model included year as a fixed factor and the lipid to protein ratios of the diets

were used as covariates. In Year 2, the average contribution was estimated as the average of the contributions for each of the time intervals. The estimated contribution (C_l) of lipid to metabolic demand was estimated for the average fish in each diet group by the equation:

$$C_l = \frac{\left(\%L_o \times DM_o - \%L_f \times DM_f\right)}{E_o - E_f} \qquad \text{(Equation 3.5)}$$

Where $\%L$ refers to the proportion of dry mass allocated to lipid in the average fish at the beginning ($\%L_0$) or end ($\%L_f$) of the fast. DM refers to the estimated dry mass of the average fish at the beginning (DM_0) or end (DM_f) of the fast and E refers to total energy content at the beginning (E_o) and end (E_f) of the fast. In both years $\%L$ was estimated by a nested ANOVA with diet as a fixed factor and sampling period nested in diet. The least square means for each combination of diet and sampling period were used to estimate $\%L$ if sampling period had a significant effect on lipid levels. If effect of sampling period was not significant then the average value for a given diet was used for both L_0 and L_f. Significance was assessed at $\alpha < 0.05$. Values for DM were estimated using ANCOVA with diet as a fixed factor and sampling period nested in diet, as previously described for total energy. Length was the covariate and a common slope was used to determine least squares estimates of DM and E for a fish 64 mm in fork length. If sampling period was found to be significant the least squares estimate for each combination of diet and sampling period was used. Variables used in the ANCOVAs were logarithmically transformed prior to analysis. The assumption that the slope relating length and DM (or

E) was parallel across all sampling strata was initially verified by inspecting all of the appropriate interaction terms. A similar approach was used to estimate C_p, the contribution of protein to metabolic demand.

Data Analysis: Energy loss over winter in wild coho salmon

ANOVA was used to examine changes in protein, lipid, dry mass and energy content of fish collected from Montana Creek in Years 1 and 2. Protein and lipid content were examined by an ANOVA using years as a main factor and days nested within years. Length-adjusted dry mass and energy were examined by a nested ANOVA with year as the main factor and sampling date nested within year and length as a covariate. Dry mass and length were logarithmically transformed prior to analysis. Untransformed data are presented to simplify interpretation. Mass-specific energy content, percent lipid and protein were examined by a similar design, but without the covariate.

Results

Effects of diet on body composition

Diet had a direct effect on body composition at the end of feeding. In Year 1 there were significant differences in the lipid and protein contents of fish fed the different diets ($F_{2,17} > 8.91$; $P < 0.002$). There was no effect of ration on these measures ($F_{2,17} < 0.64$; $P > 0.541$), consistent with observations of excess food on the bottom of all the tanks. Consequently, ration effects were dropped from all further analyses involving Year 1 fish. Beef-fed fish had the highest lipid content (Table 3.3) and chironomid-fed fish had the lowest. In turn, chironomid-fed fish had the highest protein content (Table 3.3). In

Year 2, lipid varied significantly among the groups ($F_{2,22} > 8.32$; $P = 0.002$). The fish- and beef-fed groups had the highest average lipid content, consistent with the relatively high lipid content of their diets (Table 3.4). Protein content did not differ among diet groups ($F_{2,5} = 1.90$; $P = 0.243$), but sample sizes were small. A significant and positive slope was found to relate dietary lipid to protein ratios and the resulting ratio in fish tissues ($F_{1,27} = 18.78$; $P < 0.001$) (Figure 3.1). In addition, there was difference between years ($F_{1,27} = 6.34$; $P = 0.018$) where lipid to protein ratios were generally higher in Year 1 compared with Year 2.

Effects of diet on metabolic rate

In Year 1 the chironomid-fed fish had lower metabolic rates than the fish- or beef-fed fish. The ANCOVA for total energy indicated a significant effect of sampling period on the total energy content (Table 3.5) due to the loss of energy over the 59 d fast (Table 3.3) . The ANCOVA also indicated that the total energy content of fish averaged over the entire period depended on diet, presumably due to the higher energy content of the beef-fed fish at the beginning of the fast (Table 3.3). Consequently, the estimated proportional loss of energy (p in equation 3.2) during the fast was 21.5, 23.0 and 25.0% for chironomid-fed, beef-fed and fish-fed fish, respectively. Comparison of the estimated metabolic rates of the fish fed different diets by ANOVA indicated significant differences in the metabolic rates of the different groups (Table 3.5). Chironomid-fed fish had the lowest rate averaging 14.4 ± 1 J $\cdot$ g^{-1} $\cdot$ day^{-1}, (wet weight). Beef-fed fish were intermediate with a rate of 18 ± 1 J $\cdot$ g^{-1} $\cdot$ day^{-1} and of the fish-fed fish averaged 20 ± 2 J $\cdot$ g $\cdot$ day^{-1}.

Fish lost energy over the course of the Year 2 experiment. The initial ANCOVA (equation 3.4) indicated the length adjusted energy content declined with time during the Year 2 fast ($F_{3,86} = 7.64$; $P < 0.001$). The proportionate decrease in energy (p in equation 3.2) from this ANCOVA was 7.2, 10.5 and 18.0% on days 16, 29 and 43, respectively.

In Year 2, the average metabolic rate of fish depended on their initial diet and the amount of time they fasted. The ANCOVA used to compare of the metabolic rates calculated from equation 3.2 indicated that diet had a significant effect on metabolic rate (Table 3.5). Metabolic rates of fish-fed fish averaged 17.4 ± 0.1 J · g^{-1} · day^{-1} over the three sampling periods. beef-fed and captive-wild fish averaged 18.5 ± 0.1 and 18.3 ± 0.1 J · g^{-1} · day^{-1}, respectively. Metabolic rates of the chironomid-fed fish were lowest, averaging 14.8 ± 0.1 J · g^{-1} · day^{-1}. In addition to diet related effects, metabolic rates co-varied significantly with time (Table 3.5). The slope relating length of the fast and metabolic rate was negative and differed significantly from zero ($t = 20.01$; $p < 0.001$). Thus, metabolic rate declined as the length of the fast increased (Figure 3.2).

Substrates used to meet metabolic demand

Body composition of fish changed during the Year 1 fast. Lipid content, expressed as a percentage of dry mass decreased during the fast, while protein increased (Table 3.3; Table 3.5). This indicates that the relative proportions of lipid and protein changed as mass declined during the fast. As a result, the contributions of lipid and protein to the metabolic demand of fish- and chironomid-fed fish were nearly equal, approximately 50% (Figure 3.3). In contrast, lipid catabolism accounted for nearly 62% of the metabolic demand in the beef-fed fish, while protein accounted for only 39%.

Body composition did not change during the fast in Year 2. No effect of sampling period was detected on either the lipid or protein content of the fish when these were expressed as a percentage of dry mass (Table 3.4; Table 3.5). Consequently, lipid and protein contributed to total mass and energy loss in proportion to their initial levels (Figure 3.4). This meant that lipid contributed much less to metabolic demand than in Year 1. Losses in lipid accounted for 24% of the metabolic demand in the captive-wild, beef- and fish-fed groups when averaged over the three sampling periods. Contributions of lipid to metabolic demand in the chironomid-fed fish were lower, averaging near 16%. Contributions of protein to metabolic demand ranged from 54% in the fish-fed fish to 70% in the chironomid-fed group.

The contribution of lipid to metabolic demand depended directly on the lipid to protein ratio of the diet prior to the fast. The ANCOVA indicated that the contribution o of lipid increases in proportion to the lipid to protein ratio of the fish (Table 3.6). In addition, the contribution of the lipid to metabolic demand for a given diet also depended on the year examined (Figure 3.3) (Table 3.6).

Energy loss over winter in wild coho salmon

Stream conditions varied over the two winters. During the first winter (2004-2005), stream temperatures averaged 1.0 °C during the sampling period with a range of 0 to 3.8 °C (Figure 3.4). Temperatures were highest at the beginning of winter when discharge was most variable. During the second winter (2005-2006) temperature was only measured during the fasting period. During that period water temperatures averaged 0.3 ± 0.3 °C in contrast to the previous winter when temperatures averaged 0.7 ± 0.1°C.

Comparisons of the daily discharge indicated that stream flow was much more variable during the first winter.

While coho salmon changed in body composition during both winters, they did not lose energy or dry mass. There was no difference among sampling dates in a given year for either length-adjusted dry mass or energy content ($F_{7,56} < 2.07$; $P > 0.062$). There were, however differences between years in the average dry mass ($F_{1,57} = 23.41$; $P < 0.001$) and energy content ($F_{1,57} = 22.91$; $P < 0.001$). Fish sampled in Year 2 fish were 10% heavier and had 14 % more energy than similarly sized coho salmon from Year 1 (Figure 3.5). The ability of coho to maintain size and energy content over winter indicates they must have been foraging, which was consistent with observations of prey in their stomachs. An average 75% of the stomachs examined in Year 2 had identifiable remains in them. Despite their ability to maintain mass, coho lost lipid over the winter. Lipid content as a percentage of dry mass declined until late winter ($F_{7,58} = 2.20$; $P = 0.047$) in both years and then began increasing in spring (Figure 3.5). In Year 1, lipid decreased from a high of 17% in December to a low of 14% in mid March. In Year 2 lipid decreased from a high of 17% in December to a low of 13% in late February. Despite this offset in timing the overall average lipid content of fish was the same in both years ($F_{1,58} = 0.92$; $P = 0.341$). Protein content did not change with time ($F_{6,33} = 0.28$; $P = 0.942$) nor did it vary across years ($F_{1,33} = 0.23$; $P = 0.632$).

Discussion

Diet had significant effects on body composition and metabolic rates. In Year 1 consumption of chironomids led to lower lipid levels and metabolic rates in coho salmon

relative to those that ate beef or adult salmon tissues. At the end of a 59 day fast, the fish fed salmon tissue had higher lipid content than those fed chironomids, despite having a higher metabolic rate. In Year 2, comparisons of the beef and fish diets indicated that differences in the fatty acid composition of otherwise similar diets had little impact on lipid levels and metabolic rate. Consequently, the effects of the diets observed in Year 1 likely relate to differences in their lipid to protein ratios rather than protein or lipid composition. Comparisons of the chironomid-fed fish between years indicated significant differences in the body composition and metabolic rates. These differences likely relate to differences in the water temperatures between the two years.

Temperature effects on body composition and metabolism

Comparison of the body compositions of fish fed isolipidic diets indicates relatively low temperatures optimize the storage of lipid. Water temperatures during the feeding phase of the experiments averaged 4 °C in Year 1 and 5.9 °C in Year 2. In Year 1 the chironomid-fed fish averaged 15.6% lipid at the end of feeding. In contrast, chironomid-fed fish in Year 2 averaged 10.4% lipid. Note that this discrepancy was not the result of differences in the lipid to protein levels of the respective diets. The chironomids in Year 1 fish had a lipid to protein ratio less than that of Year 2. The same phenomenon was evident by comparing the Year 1 fish-fed group to the Year 2 chironomid-fed group. Both groups received diets with lipid to protein ratios of 0.18. However, the fish in Year 1 had approximately twice the lipid of the Year 2 fish at the

end of feeding. The higher temperature in Year 2 apparently led to increased metabolic costs and a decreased ability to store lipid, despite being fed *ad libitum*.

The effect temperature on the ability to retain lipid extended to the fasting period. In Year 1 water temperature during the fast was approximately three degrees warmer than in Year 2; 3.25 °C versus 0.3 °C, respectively. Fasting fish in Year 1 changed in body composition during the fast as a result of disproportionate consumption of lipid while fish in Year 2 maintained their body composition. This difference in behavior was observed even in fish that started their respective fasts with similar body composition. For example, chironomid-fed fish in Year 1 and beef-fed fish in Year 2 both began their fasts with equal lipid to protein ratios. Despite this similarity, Year 1 fish met 50% of metabolic demand by catabolizing lipid while the Year 2 fish met 25% of their metabolic demand with lipid. In addition, the Year 2 fish that fasted at the lower temperatures ended their fast with greater reserves of lipid.

Increased loss of lipid in Year 1 indicates proportionately higher metabolic costs when fish fast at a higher temperature. The temperature effect can be determined from the temperature coefficient or Q_{10}. The Q_{10} represents the proportional change in metabolic rate over a fixed temperature change (Hochachka and Somero 2002). Decreases in metabolic rate with time in Year 2 indicate that metabolic rate was constantly down regulated during the fast, consequently rates should be compared between fish fasted for similar periods. In addition, diet-related effects indicate that comparisons should be further limited fish fed similar diets. Chironomid-fed fish in Year 1 fasted for 59 days and averaged 14.4 $J \cdot g^{-1} \cdot day^{-1}$ ($MR_{high\ temp.}$) in contrast to those in Year 2 that fasted for 43

days and averaged 9.2 $J \cdot g^{-1} \cdot day^{-1}$ ($MR_{low\ temp.}$). Water temperatures averaged 3.25 °C and 0.3 °C in Years 1 and 2, respectively. This represents a 56% increase in metabolic rate over a three degree increase in temperature or a Q_{10} equal to 4.45[5]. However, lipid to protein ratios in chironomids differed between years. A similar calculation where lipid to protein ratios are accounted for includes fish-fed fish from Year 1 with $MR_{high\ temp.}$ equal to 20.0 $J \cdot g^{-1} \cdot day^{-1}$ and chironomid-fed fish from Year 2. In this case Q_{10} is 3.08. Note that chironomid-fed fish in Years 1 and 2 had the lowest lipid levels and metabolic rates within their respective years, suggesting a role for lipid in increasing metabolic rate (Jobling 1994). The higher Q_{10} calculated for chironomid-fed fish with differing lipid to protein ratios indicates that differences in the lipid content add to the effect of temperature on metabolic rate.

Implications of metabolic rate differences

High metabolic rates in a given winter are likely to offer fish improved survival potential, despite the increased energy cost. High metabolic rates are associated with aggressive behavior and social dominance in Atlantic salmon (O'Connor et al. 2000) and rainbow trout (Cutts et al. 2002). In winter, coho salmon occupy side channels and are found at higher densities than in summer (Reynolds 1997). While aggression and dominance hierarchies are believed to be diminished during this period (O'Connor et al. 2000), stomach contents collected in Year 2 demonstrate that coho salmon are still feeding. Limited habitat and higher densities suggest competition is still an important determinant to winter survival among coho salmon. Those individuals with higher

[5] $Q_{10} = (MR_{high\ temp.} / MR_{low\ temp.})^{10/(\Delta temp)}$ (Hochacka and Somero 2002).

metabolic rates are likely to be more successful at foraging than those with low rates. For example, Atlantic salmon with relatively high metabolic rates were observed foraging more frequently in structured habitats during winter than individuals with low metabolic rate (Finstad et al. 2007).

Values obtained for metabolic rate in this study are consistent with values presented for other salmonids. Coho salmon averaging 3.3 g consumed 60 mg O_2 /kg per hour when reared at 8°C (Averett 1969). Converting that value to 19.6 $J \cdot g^{-1} \cdot day^{-1}$, using an oxycalorific equivalent of 13.59 J/mg O_2 (Jobling 1994), indicates this value is within the range of estimates (15 to 20 $J \cdot g^{-1} \cdot day^{-1}$) obtained here at 3.25°C in Year 1. The values obtained in Year 1 are close to those predicted for sockeye salmon (*O. nerka*) at 3°C (14 $J \cdot g^{-1} \cdot day^{-1}$) (Brett 1976). The Year 1 values all fall within the range, but at the high end of values reported for Atlantic salmon rearing at nearly the same temperature, 3°C (Finstad et al. 2004).

The low metabolic rates of chironomid-fed fish were not likely the result of physiological stress. Moles et al. (1997) starved coho salmon for 150 days at 4 °C and observed only 15% mortality and a wet mass loss of 30%. There was a 100% survival for fish held at 0.2° C. In Year 1, chironomid-fed fish fasted for 59 days at 3.25°C and lost only 10% of their wet mass with no mortality. In Year 2 they fasted for 43 days at an average temperature of 0.35 °C with no weight loss or evidence of mortality. Lipid content of these fish never fell below 1.39% (wet mass) in either year, which exceeds the value identified as the survival threshold in rainbow trout (0.9%) (Biro et al. 2004). While the lowest mass-specific energy content observed for Chironomid-fed fish 2.39

kJ/g (wet mass) was lower than the threshold for survival (4.6 kJ/g) reported for Atlantic salmon (Finstad et al. 2004) this reported threshold was equal to median value observed among all coho in these experiments. Apparently the threshold energy value for coho salmon is lower than that for Atlantic salmon.

It is likely that reduced metabolic rate in chironomid-fed fish in Year 1 was a response to relatively low lipid content and increased catabolism of protein to meet metabolic demand. Many species of fish are known to down-regulate standard metabolic rates (O'Connor et al. 2000) during fasting. The biochemical composition of body tissues has been identified as a potential cause for down-regulating metabolic rate during fasting (Jobling 1994). In the case of chironomid-fed fish, reduced metabolic rate would minimize the loss of protein over the winter. Muscle tissue provides the primary source of protein for catabolism. Reduced muscle mass would place fish with high metabolic rates at a disadvantage during aggressive interactions and when escaping predators.

Low metabolic rates may appear to be adaptive during periods of resource scarcity, but down-regulating metabolic rate can have a fitness cost. Fish can reduce metabolic rate by reducing protein synthesis and turnover (O'Connor et al. 2000). These reductions will likely be acute for systems that are not directly associated with meeting metabolic demand, such as immune function. For example, starved coho salmon had reduced resistance to *Renibacterium* (Moles et al. 1997) and starved catfish *(Ictalurus puctatus)* were more susceptible to *Flavobacterium* (Shoemaker et al. 2003). Reducing the standard metabolic rate can offer an advantage in situations in which there is no food available for long time periods or when food is limited and temperatures rise (Connolly

and Petersen 2003). This is only likely to happen in extreme situations because fish will defend their energy reserves by foraging even in cold water (Bull et al. 1996; Metcalfe and Thorpe 1992).

Energy loss over winter in wild coho salmon

The lipid lost by wild fish during winter represents the additional costs associated with activity associated with foraging. Wild fish in Year 2 changed body composition by losing lipid during the same period when Captive-wild fish lost energy but maintained body composition. Wild fish in Year 2 maintained their energy content during winter indicating they consumed at least an average 18.2 $J \cdot g^{-1} \cdot day^{-1}$; the average metabolic rate of the Captive-wild fish. Assuming an insect averages 3.4 kJ/g wet mass (Higgs et al. 1995), a 2 g coho salmon would have to eat at least 10 mg of insects per day in order to meet metabolic demand. The increased activity associated with this foraging apparently imposed a higher metabolic cost resulting in disproportionate lipid consumption.

It is possible that wild fish sampled in late November were already at an energy deficit, despite the presence of food in their stomachs. Lipid content of coho salmon sampled from other nearby streams in early October 2001 averaged 4.04% (wet mass) (unpublished data) and more than the peak levels observed here in late November. Increased energetic demand in early winter is consistent with observations of Cunjak (1988) that acclimation to cold temperatures is energetically demanding. In addition, extreme discharges during early winter contribute to energy demand in early winter (Cunjak et al. 1998). It is therefore possible that juvenile coho salmon obtained surplus

energy after the arrival of carcasses in late summer and then began a period in which foraging served to forestall starvation instead of supporting growth.

The winter foraging behavior of wild coho salmon in Montana Creek differed between Years 1 and 2. Fish in Year 1 lost lipid between December and March 15 at which time lipid levels fell to a minimum, averaging 13.8% (dry mass). In Year 2, fish lost lipid and energy between December and February 22 and lipid levels fell to 13.4% of dry mass. Increases in lipid following these dates indicate that energy intake surpassed metabolic demand. The difference in the timing with which foraging began means that increasing day length was not the only factor in stimulating foraging (Reinhardt and Healey 1999). The differences in the onset of foraging may relate to the minimum lipid level observed in both years. This is consistent with their need to initiate foraging to defend energy levels (Bull et al. 1996; Metcalfe and Thorpe 1992). It also suggests that 13.5% lipid (dry mass) is likely a threshold value for increased foraging in winter. This threshold value is consistent with the average levels observed among coho salmon actively foraging in Sandy Creek near Vancouver Island in July and September (Mason 1976).

Lipid-rich diets have a direct effect on body composition and metabolism, but the degree to which these effects benefit fish depends on temperature. Increases in metabolic rate result in increased lipid catabolism during growth or fasting. In this study increases in metabolic rate resulted from increased activity or elevated temperatures. Fish fasting at relatively high temperatures can respond to elevated metabolic demand by foraging or down-regulating metabolic rate. Both responses have fitness costs. Foraging increases

risk of predation and further increases metabolic demand, while down-regulating metabolic rate reduces competitive ability and may impair immune response. Carcass tissues offer fish a high quality diet in fall as stream temperatures are falling affording them the opportunity to optimize their energy reserves prior to winter. This benefit derives from the relative abundance of lipid in carcass tissues and eggs. The fatty acid composition of the lipid does not appear to be important. Thus, increased lipid content in prey is likely to be as beneficial as increased availability of carcass tissues and eggs. During relatively warm winters, the lipid obtained in these diets buffers fish from having to down-regulate metabolic rates and forestalls the need to forage. During cold winters the period in which fish can maintain high metabolic rate and minimize risk of predation is prolonged. In addition, fish that consume high quality diets in fall can start spring with elevated lipid reserves. These reserves would likely allow them to maintain high metabolic rates and hence gain a competitive advantage over those fish that failed to obtain lipid rich food the previous fall.

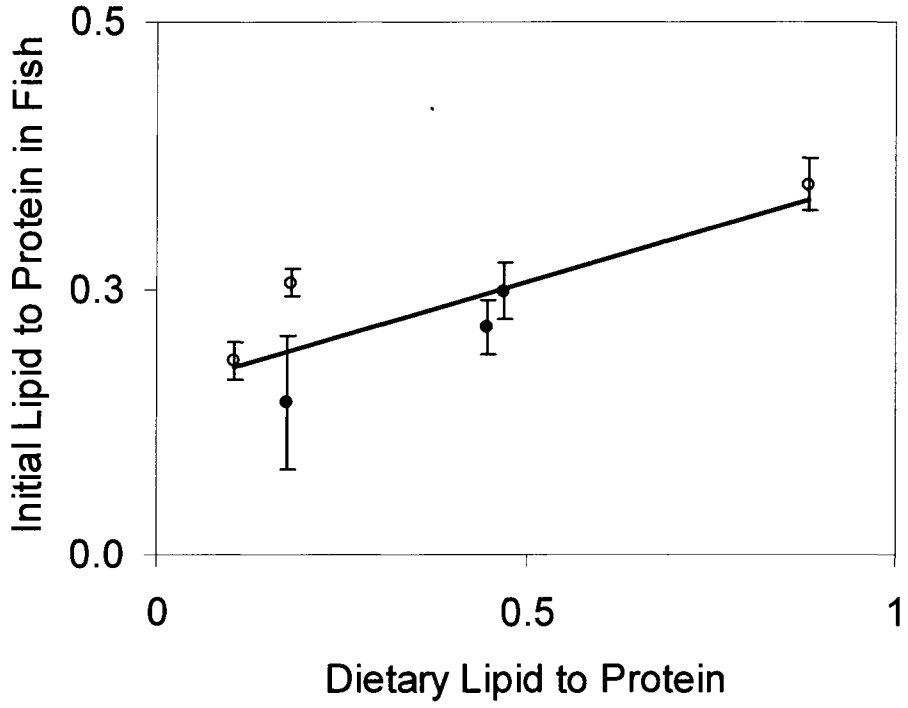

Figure 3.1. Dietary lipid to protein ratio and the resulting ratio in fish tissue. Fish fed different diets for at least 60d. Open symbols depict mean lipid to protein ratio (± 1 s.e.) of fish fed in Year 1; closed symbols : Year 2. The line depicts the common slope found by ANCOVA with Years as the main factor.

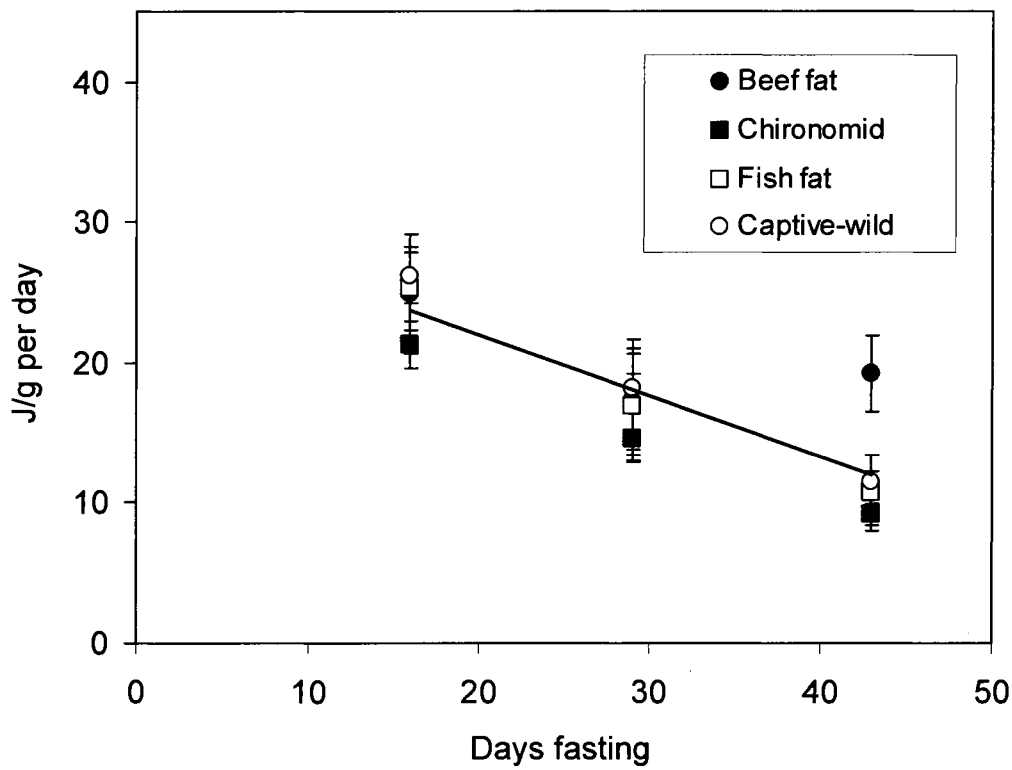

Figure 3.2. Mean metabolic rates of coho salmon in Year 2. Symbols show 95% confidence intervals for coho salmon initially fed different diets.

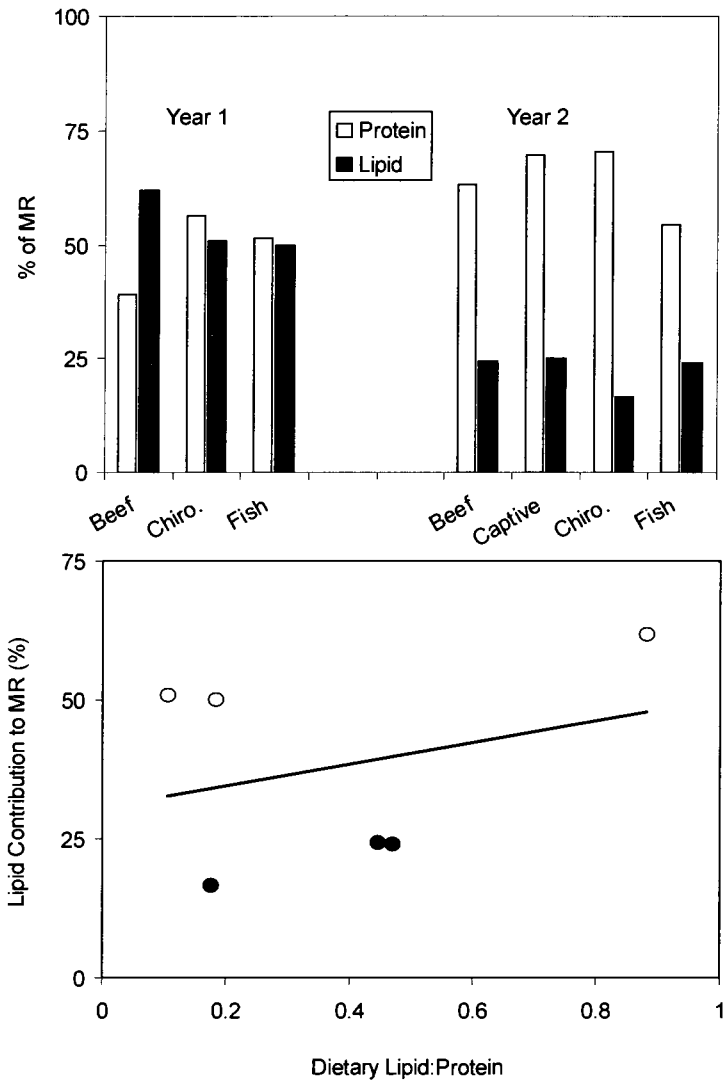

Figure 3.3. Effect of diet on substrates used to meet metabolic demand. Top Panel: Relative contributions of protein and lipid catabolism to metabolic demand during fasting in fish fed different diets in Years 1 and 2. Bottom Panel: Relationship between dietary lipid to protein ratio and the relative contribution of lipid to metabolic rate (MR) expressed as a percentage of total metabolic cost. Line shows slope of relationship common to both years. Abbreviations: Chiro. = Chironomid-fed fish; Captive = Captive-wild or fish consuming a natural diet. Open symbols: Year 1; Closed symbols: Year 2.

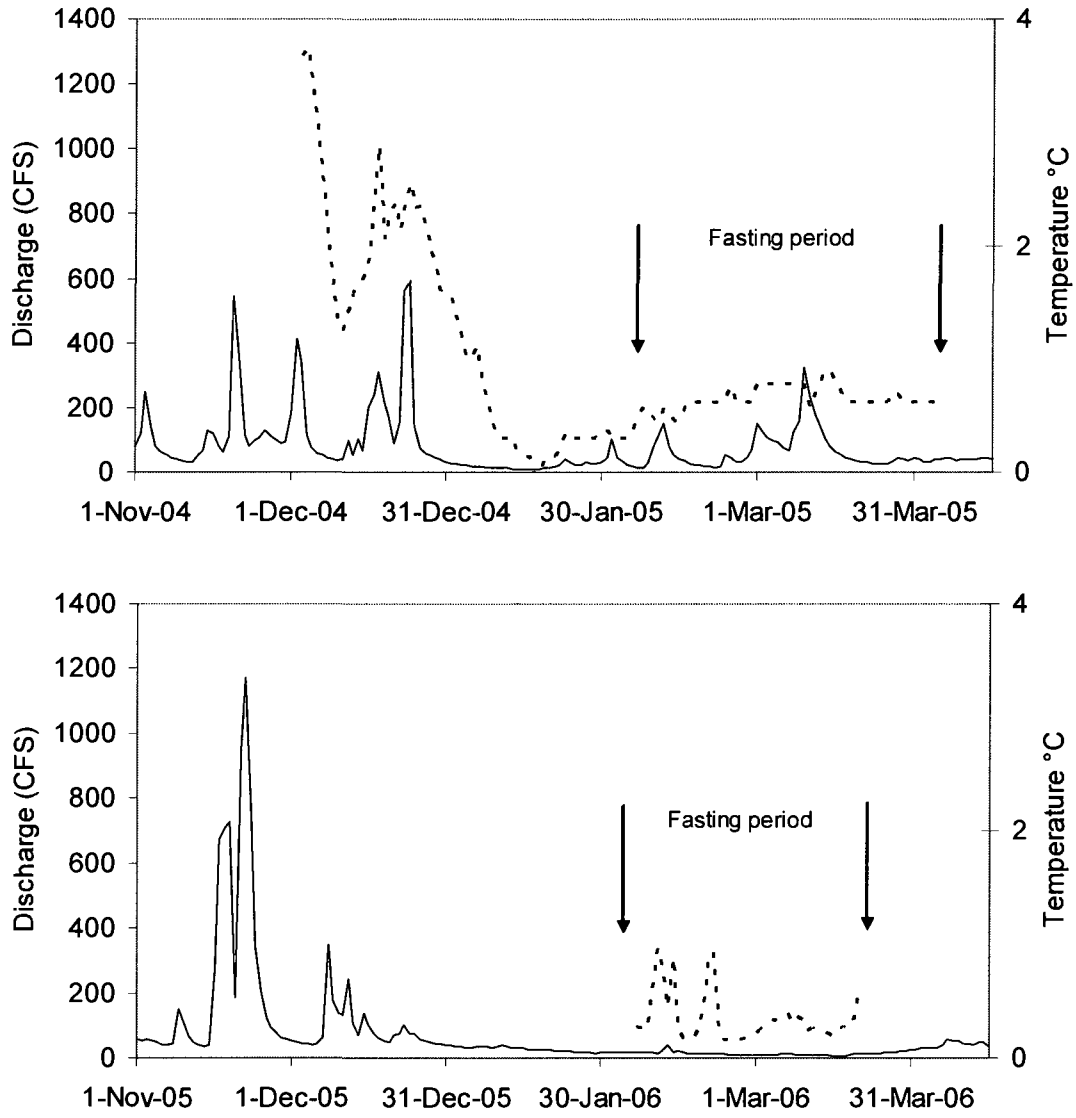

Figure 3.4. Stream discharge and temperature for Years 1 and 2. Temperature is shown as the dotted line, discharge (cubic feet per second) is solid.

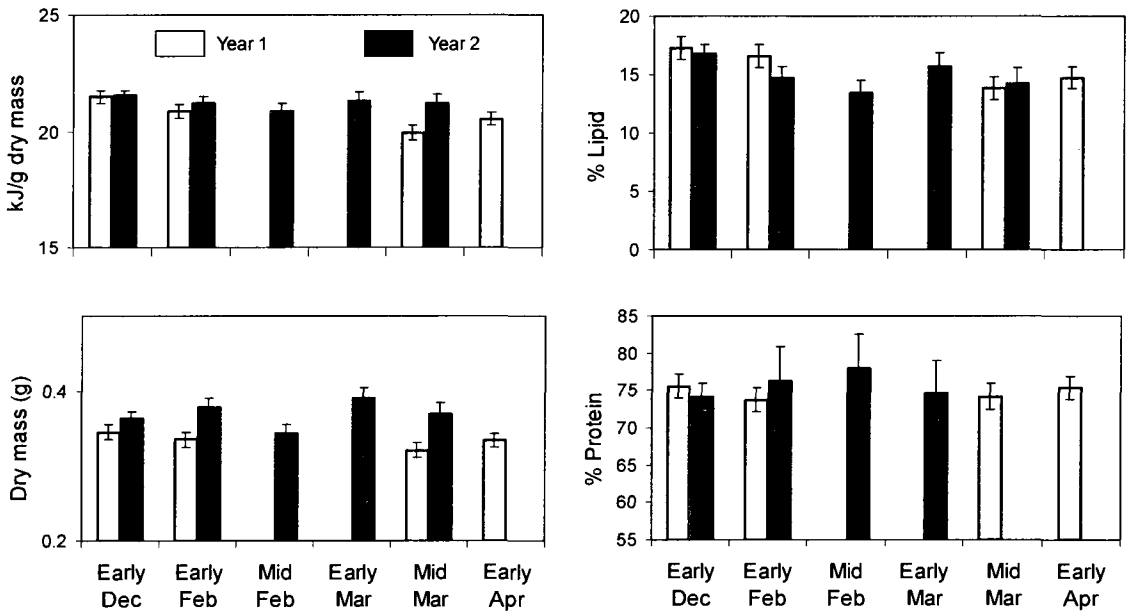

Figure 3.5. Body composition of wild fish in Years 1 and 2. Average (± 1 s.e.) mass specific energy content (top left), dry mass (bottom left), percent lipid (top right) and protein (bottom right) in wild fish collected from Montana Creek at different times during the winters of Years 1 (open bars) and 2 (closed bars). Energy, lipid and protein are shown relative to dry mass. Dry mass is displayed for a fish with length equal to 55 mm fork length.

Table 3.1. Composition of beef-fat and fish-fat diets offered in Year 2

Ingredient	Beef fat diet	Fish fat diet
Textured vegetable protein	10g	10g
High gluten wheat flour	40 g	40 g
Soy lecithin	0.3 g	0.3 g
Gelatin binder	15 g	15 g
Vitamin mix	1.46 g	1.46 g
Beef tallow	20 g	0 g
Primrose oil	4 g	0 g
Salmon oil	0 g	24 g
Ground pink salmon[1]	0 g	40.8 g
Water	54 ml	54 ml

1. The ground pink salmon contained approximately: 3.2 g fat, 7.8 g protein and 30 g water.

Table 3.2. Diets offered to fish in Years 1 and 2. Data shown as percent dry mass. In Year 1 ration was offered to 18 fish with initial weight of approximately 2.5 g. In Year 2 rations were offered to 30 fish with initial weight averaging 2.6 g.

	Daily ration ($kJ \cdot day^{-1}$)	% Lipid	% Protein	% Ash	% Carb.[1]	$kJ \cdot g^{-1}$	Lipid / Pro
Year 1							
Beef	High: 12.5 Low: 5.7	45.11	51.13	2.80	0.95	26.88	0.88
Fish	High: 9.6 Low: 4.1	14.46	79.04	12.17	0.00	21.15	0.18
Chir	5.0	6.18	58.70	35.12	0.00	14.05	0.11
Year 2							
Beef	57.5	24.33	54.53	2.80	18.34	22.96	0.45
Fish	53.1	29.24	62.32	2.92	5.52	24.12	0.47
Chir	38.1	10.50	59.38	0.00	30.12	20.91	0.18

1. Carbohydrate is estimated as the difference between 100 and the sum of protein, lipid and ash. Note, ash not measured for chironomids in year 2.

Table 3.3. Mean size and body composition of fish in Year 1. Chir = Chironomids. Length-adjusted values represent dry mass (d.m.) and energy content of a fish 64 mm in fork length. Values show mean (± 1 s.e.).

	Diet	N	Length (mm)	Length-adjusted dry mass (g)	Length-adjusted energy (kJ/fish)	% Lipid (d.m.)	% Protein (d.m.)
Begin Feb. 1	Beef	10	63.7 ± 2.7	0.58 ± 0.1	12.7 ± 0.5	23.1 ± 1.4	66.3 ± 1.1
	Chir	5	65.6 ± 3.1	0.54 ± 0.1	11.1 ± 0.6	15.6 ± 1.8	73.8 ± 1.5
	Fish	8	68.3 ± 3.3	0.59 ± 0.1	12.5 ± 0.6	19.4 ± 1.4	69.5 ± 1.2
End Apr. 5	Beef	22	61.6 ± 1.4	0.46 ± 0.1	9.7 ± 0.3	18.1 ± 0.9	71.1 ±0.7
	Chir	11	65.1 ± 2.2	0.43 ± 0.1	8.7 ± 0.4	11.6 ± 1.2	75.7 ± 1.0
	Fish	22	66.4 ± 1.4	0.45 ± 0.1	9.4 ± 0.3	16.1 ± 0.9	74.2 ± 0.7

Table 3.4. Mean size and body composition of fish in Year 2. Values show mean (± 1 s.e.), length-adjusted estimates of c dry mass and energy represent a fish 64 mm in fork length. When errors are not provided only one sample was processed. Missing values indicate no measurements were made. Chir = Chironomids

	Diet	N	Length (mm)	Length-adjusted dry mass (g)	Length-adjusted energy (kJ/fish)	% Lipid (dry mass)	% Protein (dry mass)
Feb 6 Day 0	Beef	8	57.7 ± 4.4	0.6 ± 0.1	12.4 ± 0.4	15.0 ± 1.3	74.0 ± 0.1
	Captive	9	64.1 ± 5.0	0.6 ± 0.1	12.3 ± 0.4	15.1 ± 0.8	74.9 ± 1.4
	Chir	9	60.4 ± 4.1	0.5 ± 0.1	10.7 ± 0.3	11.5 ± 1.0	78.6 ± 3.1
	Fish	8	62.5 ± 4.7	0.6 ± 0.1	14.3 ± 0.5	18.1 ± 1.1	72.2 ± 2.1
Feb 22 Day 16	Beef	5	63.4 ± 8.0	0.6 ± 0.1	11.6 ± 0.5	14.6 ± 2.7	70.6
	Captive	5	59.4 ± 4.1	0.6 ± 0.1	12.0 ± 0.5	15.9 ± 1.7	78.1
	Chir	5	61.6 ± 2.8	0.5 ± 0.1	9.7 ± 0.4	11.4 ± 0.8	81.8 ± 0.8
	Fish	4	60.5 ± 4.5	0.6 ± 0.1	13.0 ± 0.6	19.4 ± 2.5	72.4 ± 0.9
Mar 7 Day 29	Beef	3	56.0 ± 4.6	0.5 ± 0.1	11.6 ± 0.6	16.7 ± 1.5	75.0
	Captive	5	63.8 ± 3.5	0.6 ± 0.1	11.9 ± 0.5	15.6 ± 1.7	76.5 ± 0.9
	Chir	5	65.6 ± 6.7	0.5 ± 0.1	9.4 ± 0.4	9.7 ± 0.5	81.1 ± 0.6
	Fish	4	58.0 ± 4.1	0.5 ± 0.1	11.7 ± 0.5	18.8 ± 2.1	78.5

Table 3.4, continued.

	Diet	N	Length (mm)	Length-adjusted dry mass (g)	Length-adjusted energy (kJ)	% Lipid (dry mass)	% Protein (dry mass)
Mar 21 Day 43	Beef	1	50.0	0.5	10.1 ± 1.0	15.6	
	Captive	10	63.2 ± 4.4	0.5 ± 0.1	11.0 ± 0.3	14.4 ± 1.0	79.2 ± 0.9
	Chir	7	62.4 ± 4.6	0.4 ± 0.1	8.5 ± 0.3	9.7 ± 0.6	79.6 ± 1.0
	Fish	3	56.7 ± 5.4	0.5 ± 0.1	11.4 ± 0.6	15.9 ± 2.7	74.4

Table 3.5. Results of ANOVAs and ANCOVAs used to estimate metabolic rate.

	Diet		Period	
Response variable	F	*P*	F	*P*
	Year 1			
% Lipid[a]	$F_{2,69} = 13.08$	< 0.001	$F_{3,69} = 5.37$	0.002
% Protein[a]	$F_{2,70} = 15.63$	< 0.001	$F_{3,70} = 8.95$	< 0.001
Dry mass[b]	$F_{2,68} = 2.78$	0.069	$F_{3,68} = 40.73$	< 0.001
Total energy[b]	$F_{2,65} = 4.14$	0.020	$F_{3,65} = 23.72$	< 0.001
Metabolic rate[c]	$F_{2,47} = 14.3$	< 0.001		
	Year 2			
% Lipid[a]	$F_{3,75} = 18.47$	<0.001	$F_{12,75} = 0.51$	< 0.902
% Protein[a]	$F_{3,18} = 10.60$	<0.001	$F_{11,18} = 1.40$	0.253
Dry mass[b]	$F_{3,74} = 15.07$	<0.001	$F_{12,74} = 3.61$	< 0.001
Total energy[b]	$F_{3,74} = 24.46$	<0.001	$F_{12,74} = 3.35$	0.001
Metabolic rate[d]	$F_{3,52} = 11.49$	<0.001	$F_{1,52} = 400.5$	< 0.001

a. ANOVA: Response = Diet + Sampling period (Diet)

b. ANCOVA: Log(Response) = Log(Length) + Diet + Sampling period (Diet)

c. ANOVA: MR = Diet

d. ANCOVA: MR = Diet + Sampling period

Table 3.6. ANCOVA of effects of diet on lipid contribution to metabolic demand. In the model, lipid to protein ratio was a covariate and year was a random main factor.

Response	Source	DF	Mean square	F	*P*
Contribution of lipid	Diet L:P	1	0.0111	35.65	0.009
	Year	1	0.1542	494.4	<0.001
	Error	3	0.0003		

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Appendices

Appendix 3.1. ANOVA results for tests on body composition. Salmon fed different diets and rations in Year 1 and different diets in Year 2. In all cases, diet was a fixed factor. In Year 1 rations were nested within diets. Body composition was examined by ANCOVA, with dietary lipid to protein ratio as the covariate and year as a fixed factor.

Response	Source	DF	Mean square	F
Year 1- % Lipid[a]	Diet	2	0.09038	8.91
	Ration (Diet)	2	0.00064	0.64
	Error	17	0.00101	
Year 1- % Protein[a]	Diet	2	0.00088	15.54
	Ration (Diet)	2	0.00022	0.39
	Error	17	0.00057	
Year 2- % Lipid[b]	Diet	2	0.0091	8.32
	Error	22	0.0011	
Year 2- % Protein[b]	Diet	2	0.0027	1.90
	Error	22	0.0014	

Appendix 3.1 continued.

Response	Source	DF	Mean square	F
Body Composition[c]	Year	1	6.09	0.001
	Lipid:Protein	1	17.69	0.020
	Error	27		

a. Model: Response = Diet + Ration (Diet)

b. Model: Response = Diet

c. Model: Response = Dietary L/P + Year

Appendix 3.2. ANOVA and ANCOVA results for wild fish. Coho salmon collected from Montana Creek in the winters of 2004-2005 and 2005-2006. Tests of dry mass and total energy used length as a covariate. d.m. = dry mass.

Response	Source	DF	Mean square	F
% Lipid (d.m.)[a]	Year	1	0.001	0.92
	Sampling date	7	0.002	2.20
	Error	58	0.001	
% Protein (d.m.)[a]	Year	1	0.001	0.23
	Sampling date	6	0.001	0.28
	Error	33	0.002	
Dry mass[b]	Length	1	2.15	1858
	Year	1	0.03	23.4
	Sample date	7	0.02	1.66
	Error	57	0.001	

Appendix 3.2 continued

Response	Source	DF	Mean square	F
Total energy[b]	Length	1	2.12	1075
	Year	1	0.04	22.9
	Sample date	7	0.004	0.062
	Error	56	0.002	

a. Model: Response = Year + Sampling date (Year)

b. Model: Response = Length + Year + Sampling date (Year)

Chapter IV

Energy allocation strategies of resident Dolly Varden (*Salvelinus malma*) in anadromous and non-anadromous streams[6]

Abstract

When adult salmon return to their natal streams to spawn they deliver energy in the form of carcass tissues and eggs. This energy is directly available to juvenile salmonids that consume marine-derived tissues or indirectly available through bottom-up processes. Currently, the effect of marine-derived energy on the growth and energy allocation strategies of juvenile salmonids is unknown. This project contrasted the growth and energy allocation strategies of stream-resident Dolly Varden (*Salvelinus malma*) in pairs of anadromous and non-anadromous streams from two different regions of Alaska; one near Homer the other near Juneau. Streams in the two regions differed in geomorphology and inherent nutrient levels. Fish were sampled monthly during the summer of 2004 to determine growth, proximate and fatty acid compositions. Growth and lipid levels of Dolly Varden were highest in the Homer area streams, which had relatively high nutrient levels. Within regions, growth and lipid levels were higher in fish from the non-anadromous streams. Consequently, Dolly Varden residing in streams receiving marine subsidies were in poorer nutritional condition than those without subsidies. Fatty acid analysis of these Dolly Varden and adult salmon tissues indicated that resident Dolly

[6] Heintz, R. A. Energy allocation strategies of resident Dolly Varden (*Salvelinus malma*) in anadromous and non-anadromous streams. Prepared for submission in Transactions of the American Fisheries Society.

Varden in anadromous streams acquired marine-derived lipids. Most individuals acquired these lipids through indirect processes. Examination of seasonal changes in growth and energy allocation indicated that most of the energetic subsidies enjoyed by resident Dolly Varden were either transitory or delayed until carcasses were gone from the system. The few individuals that directly acquired marine-derived lipids had higher average RNA/DNA ratios and lipid levels than conspecifics that relied on indirect acquisition. These data indicate that direct consumption of marine-derived lipids has a substantive effect on energy allocation strategies in resident Dolly Varden, but few individuals make use of this resource.

Introduction

The presence of adult salmon carcasses in fluvial habitats represents an important energy resource to juvenile salmonids, but little is known about how juveniles exploit that energy. Juvenile salmonids must balance their need for growth against the need to store energy for winter (Post and Parkinson 2001) and the balance they strike influences the timing of life history transitions (Thorpe 1990). These issues are particularly important for juvenile salmonids, whose small size makes them susceptible to multiple of predators, and their relatively high mass-specific metabolic rates make them more susceptible to starvation (Schmidt-Nielsen 1984). The energy offered by adult salmon carcasses may be an important feature of juvenile salmonid habitats. The presence of adult carcasses has been shown to both increase growth (Wipfli et al. 2003) and energy reserves (Heintz et al. 2004) in juvenile coho salmon under controlled conditions. Increased size and energy reserves in juvenile fish at the end of the growing season lead to increased over-winter survival (Schultz et al. 1998). Consequently the presence of adult carcasses in fluvial habitats may have important implications for the production of salmonids from those habitats.

The relationship between carcass density and energy allocation in juvenile salmonids integrates the effects of marine-derived nutrients on watersheds. Juvenile salmonids are high level consumers and thus occupy a pivotal position in the foodwebs of Alaskan watersheds. Growth and energy storage in juvenile salmon integrate the effects of abiotic features as well as the availability of prey and the relative predation risk (Post and Parkinson 2001). The presence of carcasses increases the availability of prey by

increasing their mass and density in receiving streams (Wipfli et al. 1999) or by providing tissue that can be consumed (Heintz et al. 2004). The increased availability of prey and tissue may also potentially reduce predation risk. Conceptually, the overall impact of marine-derived nutrients on resident fish populations can be estimated by measuring the growth and energy storage of fish exposed to different levels of marine inputs.

The primary difficulty in measuring the effects of marine-derived nutrients on fish growth is that most of the fish in these systems are themselves anadromous and therefore are not found in streams without salmon runs. This obviates direct comparison of growth in different stream types. To date, experiments have consequently involved either mesocosm (e.g. Wipfli et al. 2003) studies where anadromous species are exposed to varying carcass densities, or artificial supplementation of streams that have no anadromous populations (e.g. Wipfli et al. 2004). The former approach involves removing juvenile salmonids from their natural habitat and placing them in artificial conditions. The reliance of the latter approach on streams without anadromous populations means that the fish communities are less diverse than those that occur in streams with anadromous populations. An alternative approach is to contrast seasonal changes in growth and energy allocation among conspecific populations of some non-anadromous species found in streams with and without salmon runs.

The goal of this study is to determine how the presence of adult salmon carcasses influences seasonal patterns of growth and energy allocation in Dolly Varden char (*Salvelinus malma*). In particular, this study focuses on the resident form of Dolly Varden

(Armstrong and Morrow 1980). Resident Dolly Varden spend their entire lives in freshwater and are found in anadromous and non-anadromous streams throughout Alaska. In this study, Dolly Varden were sampled monthly during the ice-free months from two streams on the Kenai Peninsula and two streams near Juneau, in southeastern Alaska. The objectives were to characterize differences in the growth and energy allocation of Dolly Varden in systems with (anadromous) and without (barriered) marine subsidies and verify their use of marine-derived nutrients by examining the fatty acid compositions of the fish before and after carcasses arrived. Growth was monitored by estimating length-at-age and measuring the ratio of RNA to DNA (RNA/DNA) in muscle. Energy allocation was determined by measuring the protein and lipid content of individual fish and determining the relative contributions of these substrates to energy content. Verification of marine lipids in Dolly Varden tissues relied on comparisons of their fatty acid compositions with those of adult salmon that returned to the streams.

Methods

Study locations and sample collection

Samples were collected from two pairs of streams to reduce the potential confounding effects of underlying nutrient levels and stream size. One pair of streams was located on the west side of the Kenai Peninsula, near Homer Alaska (Figure 4.1) and the other near Juneau (Figure 4.2), in southeastern Alaska. Pairs were selected to ensure that streams experienced similar climates, land use, geomorphology and water chemistries. Streams on the Kenai Peninsula meander through formations composed of

uplifted seabeds consisting of layered sand, silt, clay, conglomerates and volcanic ash. In contrast, the streams in southeastern Alaska are relatively short and steep, cutting through granitic bedrock and glacial till. These differences in geomorphology cause higher levels of phosphorous in the Homer streams relative to those in Juneau (Table 4.1). Within these regions streams were further selected to remove the potential effects of stream size. In the pair selected near Homer the anadromous stream had higher discharge, in the pair near Juneau the barriered stream had higher discharge

Samples from the Homer streams were collected from the Chakok River (59.8N 151.7W) and Happy Valley Creek (59.9 N 151.7 W). Both streams arise from groundwater and snowmelt on the western side of the Kenai Peninsula. The Chakok River receives marine subsidies from returning coho (*Oncorhynchus kisutch*), chinook (*O. tshawytscha*) , pink (*O. gorbuscha*) and steelhead salmon (*O. mykiss*). Unidentified sculpins and anadromous and resident Dolly Varden rear or reside there. The chinook salmon spawn in mid July through early August, a much smaller pink salmon run spawns in late August and coho spawn in mid September. A fish counting weir located at the mouth of the Chakok River in 2004 provided counts of adult chinook and coho salmon returning to the system. Happy Valley creek has resident Dolly Varden and threespine sticklebacks (*Gasterosteus aculeatus)*. A barrier at the mouth prevents immigration of anadromous forms.

Dolly Varden were collected monthly from the Homer streams between May and August 2004 and again in October 2004 and in May 2005. Dolly Varden smolts or obvious anadromous types, as indicated by silvering and deep bodied morphologies, were

not retained for analysis to ensure that comparisons were limited to resident forms from both systems. Samples were collected at fixed sampling stations 250 m in length with seven baited minnow traps fished for up to 24 h. Sampling stations were arrayed along the length of each stream (Figure 4.1). In addition five adult female chinook salmon were collected at the weir to use as fatty acid archetypes. They were divided into soma and eggs prior to analysis.

Dolly Varden recovered in traps were transported live to the lab at the Kachemak Bay Research Reserve for initial processing. In the lab fish were killed, their lengths and weights recorded, and stomachs removed. Each fish was then placed in a freezer and held at -70°C until they underwent chemical analysis at NOAA's Auke Bay Laboratory.

Samples from the Juneau area were collected from Shrine (58.4N 134.8 W) and Upper Sheep Creeks (58.2N 134.3W). Both streams are on the Juneau road system and arise from groundwater and snowmelt. Shrine Creek is the anadromous stream with resident coho, pink and chum salmon (*O. keta*), cutthroat trout (*Salmo clarkii*), and Dolly Varden. Chum salmon spawn in early August, pink salmon in mid August and coho salmon in October. In terms of biomass, the largest of these runs is the chum run. Adult salmon were enumerated by weekly foot surveys. Upper Sheep Creek is a barriered section of an anadromous stream. The upper section has resident Dolly Varden and threespine stickleback. No fish were collected from the anadromous portion of Sheep Creek.

Sampling from Juneau streams focused on age-0 Dolly Varden, so fish were only found between June and October 2004, despite attempts to collect in May. Samples of

age 1+ were collected with baited minnow traps at fixed stations along the entire length of each stream. Age-0 fish were caught by turning over rocks and aspirating exposed fish into large bore pipettes or trapping them with aquarium nets. Collections of age-0 were made monthly throughout the entire length of each stream (Figure 4.2). All fish were transported alive to the Auke Bay laboratory where they were held until processing. Generally, age-0 fish were too small to process individually for proximate composition, consequently individuals were pooled to create samples that met our minimum mass criteria of 1.0 g for analysis. Individual lengths and weights were recorded for all fish prior to pooling. The fatty acid compositions of three female chum salmon collected from the anadromous portion of Sheep Creek during a previous study were used as marine archetypes for the Juneau streams.

Aging

Fish were aged to determine if size-at-age differed between anadromous and barriered streams. The saggital otoliths from each fish were removed for aging immediately prior to chemical analysis. Otoliths were mounted on glass slides with sulcus side down and polished with a series of fine grit sandpapers. Periodically during grinding, the otolith was examined under a dissecting microscope to determine when sufficient material had been removed to reveal the annuli. The right otolith was used for each fish, if possible. Ages were recorded as the total number of annuli detected. Ages were cross-validated by having the same reader re-examine the otoliths without any identifying information. Discrepancies were resolved when an independent reader and the original reader were able to reach consensus. No ages were obtained from fish in the May

2005 sample from the Homer streams. In addition, no ages were collected from age-0 Dolly Varden collected from Juneau, instead age was assumed based on the size of individuals relative to their location in size frequency distributions.

RNA/DNA, proximate, lipid class and fatty acid analysis

Chemical analyses included estimation of the ratio of RNA to DNA (RNA/DNA), total lipid, water, ash and protein content along with analysis of the fatty acid composition. Carbohydrate content was considered negligible. Prior to homogenizing fish for proximate analysis a 100 to 200 mg piece of epaxial muscle was removed to estimate RNA/DNA. This represented less than 3% of wet mass of the fish older than age-0 and was therefore assumed to have a negligible effect on the remaining analyses. Subsequent analyses used homogenates made from the remainder of the whole fish following the procedures outlined in Vollenweider (2005). It was necessary to pool samples of age-0 fish for RNA/DNA and proximate analysis. Epaxial muscle was obtained by sampling the distal portion of the fish after cutting the fish along a line posterior to the vent and dorsal fin. The remainder of the fish was discarded. Consequently, RNA/DNA samples of age-0 fish were not matched with proximate analyses as was the case for older fish.

RNA/DNA ratios were determined spectrophotometrically from four analyses of each muscle sample; two analyses to determine DNA content and two to determine the RNA content. The ratio was estimated from the mean of each of these samples. The muscle tissue was minced and ground into a homogenate in a mortar and pestle with a known volume of water. Nucleic acids were precipitated from the tissue in $HClO_4$ and isolated by centrifugation using the following method. Four chilled snap-cap tubes were

each filled with cold 600 µl 0.27 N $HClO_4$. A 200 ul aliquot of homogenate was added to each tube and the mixture incubated on ice. After 10 minutes the mixture was spun at 10,000 × g for 5 minutes at 4°C and the supernatant was discarded. The pellet was mixed with 500 µl of 0.2 N ice-cold $HCl0_4$, vortexed and respun as before. The supernatant was discarded and pellet retained.

For determination of RNA content, two of the pellets were re-suspended in 500 µl 0.3N KOH and incubated at 37°C for 30 minutes. After incubation, 250 µl of 1.5 N $HClO_4$ was added and the mixture incubated on ice for 10 minutes. The resulting solution was spun at 10,000 x g for 5 minutes at 4°C. The supernatant was retained and the pellet washed twice with 150 µl of ice-cold 2.0 N $HClO_4$. The supernatant was combined with the initial aliquot and the absorbance determined at 260 nm.

For determination of DNA content, each of the remaining two pellets was mixed with 300 µl 0.5 N $HClO_4$, 600 µl diphenylamine solution and 5 µl 2% acethaldehyde solution and incubated for 20 h at 25°C. The diphenylamine solution consisted of 5 g diphenylamine dissolved into 333 ml glacial acetic acid and 5 ml concentrated H_2SO_4. After incubation, the solution was spun for 3 minutes at 10,000 × g and the absorbance of the supernatant was determined at 600 nm.

Concentrations of RNA and DNA were determined from five-point standard absorbance curves developed from yeast RNA and salmon sperm DNA. Standard absorbance curves were produced immediately before analysis of samples began and at 4 additional times while samples were processed. Sample blanks were run along with each batch of 10 samples to ensure method cleanliness.

Lipid was extracted from 0.5 to 1.0 g of wet sample homogenate using a modification of Folch's method outlined by Christie (2003) in a Dionex Accelerated Solvent Extractor (ASE) 200 with 2:1 (v:v) chloroform:methanol. Extracts were washed successively with a 0.88% KCl solution and 1:1 (v:v) methanol:deionized water in a volume equal to 25% of the extract volume to remove co-extractables. Excess solvent was evaporated and percent lipid was calculated gravimetrically. Quality assurance samples included with each batch of 17 lipid samples included a blank to control for cross contamination, a reference material to determine accuracy and a duplicated sample to evaluate repeatability. References were all found to be within 10% of the expected value, and duplicated samples were always within 5% of each other.

Moisture and ash content were determined using a Leco TGA-601 thermogravimetric analyzer. Approximately 2.5 g of wet homogenate were placed in a crucible inside the instrument. The temperature was increased from ambient to 135 °C over 6 minutes and then held until repeated measurements of the mass varied by less than 5%. The temperature was subsequently increased to 600 °C and held until repeated measurements of mass varied by less than 5%. Moisture content was estimated as the difference in initial mass and the stable mass at 135 °C. Ash content was estimated as the stabilized mass observed at 600 °C. Quality assurance for moisture and ash analyses included blank and duplicated samples. If the initial sample mass was less than 2.5 g, ash weight was not determined.

Protein content was estimated from the total nitrogen content observed in a 0.1 g sample of dried homogenate. Nitrogen content of the sample was measured with a LECO

FP 528 Nitrogen Analyzer following the Dumas method in which the homogenate was combusted at 850 °C and the expelled nitrogen measured by thermal conductivity. Total mass of protein was estimated by multiplying the total nitrogen content by 6.25 (Jones 1931, Craig et al. 1978). The instrument was calibrated daily using EDTA. Quality assurance samples included with each batch of 17 samples included a blank reference consisting of pure cane sugar, and a standard reference material (SRM1546) obtained from the National Institute of Standards and Technology (NIST). In addition, all analyses were duplicated to ensure the coefficients of variation for estimated nitrogen content was less than 15%. Final quality control involved summing the estimated percentages of nitrogen, lipid, ash and water and ensuring that they fell within 1.5% of 100%.

Fatty acids in the whole lipid extracts from the Dolly Varden and the adult salmon archetypes were transesterified to fatty acid methyl esters (FAMEs) using Hilditch reagent, as described in Christie (2003) except that hexane was used instead of toluene. Prior to transesterification, 19:0 and 23:0 fatty acids were added as an internal standard and a surrogate standard, respectively. Purified FAMEs in hexane were evaporated under nitrogen to a final volume of approximately 1 mL, and a FAME internal standard (21:0) was added before injection into a Varian CP3800 gas chromatograph (GC). The GC was equipped with a 100 m Varian CP Select for FAME cyanopropyl-bonded fused silica column and operated under a ramped temperature program. Separated fatty acids were detected with a Varian Saturn model 2200 mass spectrometer operating in selective ion storage mode. Samples were processed in batches of 17 along with calibration standards and quality assurance samples. Fatty acid concentrations for each of the FAMEs were

determined using five-point calibration curves, normalized to internal standard recovery. Blank, duplicate and standard reference (NIST SRM 1946) sample spectra were used for QA evaluation. Concentrations observed for the reference material were typically within 25% of the certified values. The coefficient of variation for duplicate analyses performed within a batch was generally less than 10%. The estimated total fatty acid content of method blanks was less than 10% that of the lowest estimate for samples in a batch.

Statistical Analyses

Three analyses were performed to determine if fish growth in anadromous streams differed from that of fish in barriered streams. Initially, a hierarchical ANCOVA was performed on the lengths of fish with age > 0 using stream location, hereafter referred to as region, stream types nested within regions and collection periods nested within stream types. This approach allowed for comparison of stream types within regions and for comparison of regional differences. Age was considered a covariate and the hypotheses tested were that age-length relationships were consistent between regions and within regions the length-at-age was the same between stream types using the following model.

$$L = A + R + S(R) + M(S) + RxA \qquad \text{(Equation 4.1)}$$

Where L is the length of a fish, A is its age, R the region in which it was collected and S is the stream type nested within regions and M is the sampling period nested within stream types. A significant interaction between region and age (RxA) indicates that length and age relationships differ between regions and analysis was performed

separately for each region. If no significance was associated with the interaction the slopes of the age-length relationships were assumed to be equal in both regions. The model was subsequently re-expressed using a slope common to both regions by deleting the interaction term. The hypothesis that fish from different stream types within a region had equal lengths-at-age was examined by *ad hoc* pairwise comparisons with an overall $\alpha = 0.05$.

The second analysis of growth removed any error that might have arisen through the aging method. The observed lengths and wet masses of all the age-0 fish collected from the Juneau streams were examined by ANCOVA to determine daily growth during the summer. Linear regression models were fit to the length and weights of the fish using the number of elapsed days from the first sample as the independent variable. The regressions were blocked on stream type (S) to determine if the slopes relating days (T) differed. The model tested was:

$$L = T + S + (T \times S) \qquad \text{(Equation 4.2)}$$

In this model, L is the size of the fish (length or weight), T is the number of elapsed days and S is the stream type and TxS is the interaction. A significant interaction indicates that the slopes and hence the rate of growth differs between stream types. To maintain independence of the tests, fish collected in July were randomly assigned to either the analysis of growth between June and July or July through August. No comparison was

possible for the August to October interval, because only one age 0 fish was collected from Sheep Creek in October.

The third analysis of growth relied on RNA/DNA ratios and was independent of measurements of age or length. RNA/DNA levels were compared in a hierarchical design similar to equation (4.1), but with no covariate. The model allowed testing of the hypothesis that RNA/DNA levels of fish in anadromous and barriered streams are equal within a region. Pairwise comparisons were made on an *ad hoc* basis using Tukey's Honest Significant Difference with $\alpha = 0.05$ (Milliken and Johnson 1984). In all analyses, the assumptions of normality and homogeneity of variance were examined and the data transformed as required. Tables and figures display untransformed values, to improve interpretation.

The mass specific energy contents of fish and the allocation of that energy to structure and storage were compared between stream types. Calorific equivalents for protein and lipid were combined to determine how fish from anadromous and barriered streams allocated their energy between protein (structure) or lipid (storage). The mass specific energy content, or energy density of the fish, (*ED*) was estimated from equation 4.3.

$$ED = 20.10 \times P + 36.43 \times L \qquad \text{(Equation 4.3)}$$

Where P and L are the proportion of dry mass allocated to protein and lipid, respectively (Brett 1995). Comparisons of *ED*, P and L followed the approach outlined for equation

(4.1) except that no covariate was used. Fish can alter their mass without changing their relative allocations of mass to lipid and protein, resulting in constant values for *ED*, *P* and *L* despite changes in energy content. Consequently, ANCOVAs were also performed on the total energy content and dry mass of fish from barriered and anadromous streams. These analyses were similar to that of equation (4.1), but length was used as a covariate. Length, energy and dry mass were all transformed to their logarithms (base ten) prior to analysis in order to linearize the relationships. Analyses followed the same procedure as equation (4.1) wherein the length x region interaction was initially examined. If the interaction was not found to be significant it was deleted and the model was re-expressed with both regions sharing the same slope to test the hypothesis that length-adjusted mass or energy was the same between stream types within a region. Total energy was calculated as the product of dry mass and *ED*.

It is important to recognize that the comparisons of stream types within regions described above use values that represent the mean for a given stream averaged over the entire sampling period. This means that the analysis may mask temporal shifts in the response variables that relate to the arrival of carcasses. Thus, when sampling period was found to have a significant effect on the response variable, responses were compared between types for each sampling period. These planned comparisons used an F statistic calculated as:

$$F_{1,d} = \frac{n/2\left(\bar{x}_k - \bar{x}_j\right)^2}{MS_{error}} \qquad \text{(Equation 4.4)}$$

according to Underwood (1997), where d is equal to the degrees of freedom for the MS_{error} and $\bar{x}_k$ is the mean of the k[th] sampling period for a given stream type. Often these contrasts involved different numbers of observations; in that case the lowest number of observations was used for n.

Fatty acid compositions were used to determine if Dolly Varden consumed marine-derived lipids. Fatty acid compositions of fish from each sampling period were inspected to find a set of fatty acids with detectable masses common to all of the fish examined including the chinook salmon archetypes. This set of common fatty acids was used to calculate a dissimilarity index where the dissimilarity between each sample was determined from:

$$D_{u-U} = \left[\sum_{j<n} \sum_{i<j} \left(Ln\left(u_i / u_j\right) - Ln\left(U_i / U_j\right) \right)^2 \right]^{1/2} \qquad \text{(Equation 4.5)}$$

Where $\boldsymbol{u}$ is a vector of n fatty acids describing a Dolly Varden such that $\sum_{i=1}^{n} u_i = 1.0$ and all $u_i > 0$. $\boldsymbol{U}$ is a similarly composed vector describing another sample. The resulting value was placed in a dissimilarity matrix consisting of all the pairwise comparisons between individuals. The matrix was analyzed by non-metric multidimensional scaling (NMDS) and ANOSIM. NMDS was used to reduce the number of dimensions in the data set. Models were fit ranging from 1 to 5 dimensions, and Kruskal's stress calculated. The results of NMDS modeling were plotted to inspect relationships between samples in the reduced space identified by the NMDS. A separate NMDS model that included the marine archetypes was evaluated for each region. ANOSIM was used to examine

hypotheses regarding clusters of points on the plots. It is unlikely that the fatty acid composition of Dolly Varden would ever become statistically identical to that of the marine archetype. Thus, hypothesis testing revolved around identifying differences among clusters that appeared to be located in different parts of plot. Hypothesis tests used R, a statistic that records the difference in the average similarity of samples in different test sets with the average similarity within sets (Clarke and Warwick 1994). Values are normalized by the number of samples considered so that values near 1 indicate that sample groups are completely dissimilar and values near 0 indicate similarity. Hypothesis testing involves permutating the labels assigned to each sample to determine if the arrangement of sample labels for the observed dissimilarity matrix is different from random.

Increased similarity to the marine archetypes was assumed to indicate consumption of marine-derived lipids. It is possible that some individuals in anadromous streams could have greater similarity to the marine archetypes than others sampled at the same time. In this case, the RNA/DNA, lipid and mass specific energy content of individuals classified as similar and dissimilar were compared by Student's *t* test.

Results

Fish size

Dolly Varden varied in size among the streams, those collected near Homer were longer and heavier than those from Juneau (Table 4.2). Comparison of fish from anadromous and barriered streams within the pairs indicated that fish tended to be longer

and heavier in the barriered streams despite the tendency for wider age ranges in the anadromous streams. Examination of frequency distributions of fish lengths indicated a discontinuity in sizes among fish. Lengths of age-0 fish in the Juneau streams ranged from 25 to 66 mm while those aged > 0 ranged between 72 and 112 mm (Figure 4.3). The largest of these age-0 fish was represented by a single fish caught in Sheep Creek in October; the remaining 272 age-0 fish sampled were less than 55 mm long.

Growth: Size-at-age

Dolly Varden in barriered streams were larger at a given age than those from anadromous streams, suggesting higher growth in barriered streams. The slopes relating length and age for fish aged greater than zero varied by region ($F_{1,145} = 8.53$; $P = 0.004$), so size-at-age was examined independently for the two regions. In the Juneau streams no relationship between length and age was detected ($F_{1,32} = 2.54$; $P > 0.121$), nor was there an effect of stream type ($F_{1,32}$ 1.57; $P = 0.219$) (Figure 4.4). In contrast, length increased with age by approximately 15.9 mm/year in the Homer streams ($F_{1,113} = 57.13$; $P < 0.001$). In addition, length-at-age of fish from the barriered stream was 10% longer than those from the anadromous stream ($F_{1,113} = 10.94$; $P = 0.001$). When averaged over the whole summer, the lengths of age 2+ fish were 111 and 100 mm in the barriered and anadromous streams, respectively (Figure 4.4).

Growth: Age-0 growth rates

Age-0 Dolly Varden from the barriered stream in Juneau grew at a higher rate than those in the anadromous stream. Between June and July age-0 fish increased mass at

a greater rate in the barriered stream ($F_{1,148} = 41.49$; $P < 0.001$). Wet mass increase in the anadromous stream averaged nearly one third that of the barried stream between June and July (Figure 4.5). Between July and August this difference in growth was maintained ($F_{1,109} = 4.53$; $P < 0.035$) (Table 4.3).

Differences between streams in the daily increment in length were less extreme than those of mass. Between June and July Dolly Varden in the anadromous stream increased in length at a significantly lower rate than those in the barriered stream ($F_{1,148} = 11.74$; $P = 0.001$). Between July and August these differences disappeared ($F_{1,109} = 0.027$; $P = 0.603$). The convergence in growth rates resulted form fish in the anadromous stream stepping up growth between July and August while those in the barriered stream decreased (Figure 4.5).

Growth: RNA/DNA

Fish in anadromous streams had reduced RNA/DNA ratios relative to those in barriered streams ($F_{2,178} = 18.05$; $P < 0.001$). In Juneau, the fish in the anadromous stream had ratios averaging 2.20 versus 3.42 in the barriered stream. In Homer, RNA/DNA averaged ratios 3.38 and 2.98 in the anadromous and barriered streams, respectively (Figure 4.6). The averages differed significantly in the Juneau streams ($t = 5.60$ P < 0.001), but not the Homer streams ($t = 2.17$; $P = 0.135$). These values reflect averages pooled across the whole summer. In addition, there were differences among sampling periods ($F_{14,178} = 14.24$; $P <= 0.001$). The relatively low ratio observed among fish from the anadromous stream near Juneau was due to lower growth in the beginning of the growing season. RNA/DNA ratios were significantly lower in the anadromous

stream in June and July ($t > 4.27$; $P < 0.004$). In August and October RNA/DNA ratios increased in the anadromous stream while they remained constant in the barriered stream (Figure 4.6). Consequently, there was no difference between the stream types in Juneau ($t < 1.76$; $P > 0.952$) during these months. No differences in stream type were detected for the Homer streams except for May when RNA/DNA ratios were significantly higher in the anadromous stream. Note that RNA/DNA ratios averaged higher among fish collected near Homer than those from the Juneau streams ($F_{1,178} = 6.19$; $P = 0.009$) (Figure 4.6).

Mass specific energy content

Mass specific energy content of Dolly Varden in anadromous streams was lower than that in barriered streams and energy contents remained constant through the sampling period. Averaged over all sampling periods, the mass specific energy content was 20.6 kJ / gram in the anadromous stream in Homer compared with 21.2 kJ / gram in the barriered stream. Similarly, the average mass specific energy content of fish in the anadromous stream in Juneau (19.8 kJ / gram) was also lower than that of the barriered stream (20.7 kJ / gram). Consequently, there were regional differences in mass specific energy content ($F_{1,205} = 11.84$; $P = 0.001$) and differences between streams within regions ($F_{2,205} = 10.18$; $P < 0.001$) (Figure 4.7). However, no effect of sampling period on mass specific energy content was detected ($F_{16,205} = 1.45$; $P = 0.123$).

The higher mass specific energy content among fish from the barriered streams resulted from an increased proportion of dry mass allocated to lipid (Figure 4.7). The fish in the Homer streams averaged 16.3 and 18.1 % lipid in the anadromous and barriered streams, respectively. A similar pattern existed in the Juneau streams where fish averaged

10.7 and 14.5% lipid in the anadromous and barriered streams, respectively. Thus, the proportion of lipid allocated to dry mass was significantly higher in the Homer streams ($F_{1,205} = 39.47$; $P < 0.001$). Within regions, fish had significantly higher lipid in barriered ($F_{2,205} = 13.99$; $P < 0.001$). Differences between stream types were significant in the Juneau streams ($t = 4.78$; $P < 0.001$), but not in the Homer streams ($t = 2.27$; $P = 0.108$). Lipid has 80% more energy than protein per unit mass (Brett 1995), consequently lipid accounted for approximately 20% of the energy content of fish from the anadromous stream and 27% of that from the barriered stream in Juneau. Lipid accounted for 27% of the energy in fish from the anadromous stream and 30% of that from the barriered stream (Figure 4.7). There was no temporal change in the proportion of dry mass allocated to lipid ($F_{16,205} = 1.26$; $P = 0.228$).

A consequence of the reduced allocation of energy to lipid in the fish from the anadromous stream in Juneau was that more of their dry mass was allocated to protein (Figure 4.7). Fish in Juneau streams had higher protein levels than those from Homer ($F_{1,206} = 54.13$; $P < 0.001$) averaging 79.3 and 76.7% in the anadromous and barriered streams, respectively. Similarly, fish in the Homer streams averaged 73.0 and 72.6% protein in the anadromous and barriered streams, respectively. These differences in protein content between stream types were significant ($F_{2,206} = 6.89$; $P = 0.001$). Thus, fish from the anadromous stream near Juneau allocated 78.2% of their dry mass to protein on average, compared to 76.0% for fish from the barriered stream ($t = 3.52$; $P = 0.003$). A similar difference was observed among fish from the Homer area, but it was not significant ($t = 1.16$; $P = 0.650$). Fish from the anadromous stream allocated an

average 73.8% of their dry mass to protein compared to 72.2% in fish from the barriered stream (Figure 4.7). These differences remained constant across all the sampling periods ($F_{16,206} = 1.35$; $P = 0.172$).

Length and dry mass

Fish in the Homer area increased their dry mass with length at a lower rate than fish in the Juneau area even though Homer fish had faster growth rates. The allometric exponent relating fish length to dry mass was higher for fish from Juneau (3.369) than those from Homer (3.171) ($F_{1,235} = 14.84$; $P < 0.001$). The existence of different allometric exponents meant that different stream types had to be compared separately for each region. There was no difference in the length adjusted dry masses of fish in the different types of streams within regions (Homer: $F_{1,134} = 0.46$; $P = 0.498$, Juneau: $F_{1,99} = 0.13$; $P = 0.720$). However, there were significant temporal effects on the dry mass of fish at a given length (Figure 4.8) (Homer: $F_{10,134} = 7.54$; $P < 0.001$, Juneau: $F_{6,99} = 4.54$; $P < 0.001$). Comparisons Fish in the anadromous stream in Homer had greater length-adjusted dry masses in July and August than fish from barriered streams, but these differences were not significance (Table 4.4). Fish in the barriered stream in Juneau had significantly higher length-adjusted dry mass in August than those in the andromous stream (Table 4.4).

Length specific energy content

Even though fish from the Juneau location increased their dry mass relative to length at a higher rate than those near Homer, there was no regional difference in the

relationship relating energy content and length ($F_{1,203} = 3.51$; $P = 0.063$). The allometric exponent relating total energy to length was 3.19 in both regions. Fish collected from Homer had significantly greater length-adjusted energy contents than those near Juneau ($F_{1,204} = 19.77$; $P < 0.001$) averaged over the entire summer. There was no difference between stream types in Homer ($t = 0.45$; $P = 0.970$). A fish of given length collected from the Homer streams averaged 13% and 24% more kilojoules than those from the barriered and anadromous streams in Juneau, respectively. Fish in the anadromous stream near Juneau had significantly lower length-adjusted energy than those in the barriered stream ($t = 2.63$; $P = 0.045$). In addition, there was significant temporal variation in length-adjusted energy content (Figure 4.8) ($F_{16,204} = 5.46$; $P < 0.001$). Pairwise contrasts between stream types within each region indicated no differences in energy content between fish in the Homer streams (Table 4.5). Fish in the barriered stream in Juneau had significantly higher length-adjusted energy levels in August (Table 4.5) but this difference disappeared by October.

Dolly Varden and adult salmon fatty acid compositions

Analysis of the similarity in the fatty acid compositions between Dolly Varden in Homer and adult salmon tissues indicated that few Dolly Varden directly incorporated marine lipids (Figure 4.9). Examples of fatty acid compositions are provided in appendices 4.1-4.3. In general, fish collected from the anadromous stream near Homer had fatty acid compositions more similar to those of adult salmon tissues than those from the barriered stream. Dissimilarities between fish in the anadromous stream and the marine archetypes averaged 20.8 compared with 22.7 for fish from the barriered stream

(R = 0.218, P = 0.001). Inspection of the NMDS plot for the anadromous stream indicated that the fatty acids compositions of two individuals in August and three in October had unusually low dissimilarities to the adult salmon tissues (Figure 4.9). Dissimilarity between the fish in the anadromous stream and the adult tissues averaged 20.0 in August, while the two individuals averaged 12.5. In October the average dissimilarity was 19.1, while the three individuals averaged 12.22. Taken together, these five individuals had significantly different fatty acid compositions from the other Dolly Varden sampled at the same time (R= 0.707, P = 0.001). These individuals had higher average RNA/DNA, lipid and mass specific energy content than other Dolly Varden sampled at the same time, but the differences were not significant (t < 1.65; P > 0.159).

Few Dolly Varden directly incorporated marine lipids in the anadromous stream near Juneau. As with the comparison made between the Homer streams, dissimilarity between the Dolly Varden fatty acids and those of the marine archetypes averaged 21.6 in the anadromous stream compared with 25.4 in the barriered stream (Figure 4.9). In addition, the fatty acid compositions of the Dolly Varden in the two streams differed significantly (R = 0.639, P = 0.001). After the carcasses arrived in the anadromous stream, three individuals had significantly lower dissimilarities (R = 0.667, P = 0.001) to the marine archetype than contemporaneously sampled fish (Figure 4.9). The dissimilarity between the marine archetype and these three fish averaged 11.3 compared with 20.8 for all fish sampled in August and October. As a group, these Dolly Varden had significantly higher RNA/DNA levels (t = 3.81; P = 0.019) and higher average lipid and

mass specific energy levels than others sampled at the same time. The latter differences were not significant ($t < 1.98$; $P > 0.095$).

Discussion

Differences in the inherent geomorphology of streams were more important in determining the energy allocation strategies of resident Dolly Varden than the presence of carcasses. Dolly Varden from the phosphorous poor streams near Juneau had lower size-at-age, RNA/DNA ratios and energy content than Dolly Varden from comparatively phosphorous rich streams near Homer. In addition, the growth strategies in the two regions differed. Fish in the Homer streams increased in length faster than those near Juneau, but for a given change in length, fish in Juneau gained more dry mass. Despite differences in the rate at which fish gained mass relative to a fixed change in length, there was no difference in the rate at which energy changed over the same interval. The higher mass specific energy content of the Homer Dolly Varden compensated for their lower dry mass.

Differences in growth and mass allocation of Dolly Varden in the streams suggest a continuum of strategies for allocating ingested energy. In systems where growth is relatively rapid, changes in length coincide with increased lipid and mass specific energy. Increases in lipid and hence mass specific energy content are characteristic of salmonids enjoying rapid growth (Post and Parkinson 2001). In the systems where growth is relatively slow fish maximize their dry mass. This emphasis means that relatively slow growing fish at a given length can have mass and energy contents equal to or higher than that of fish in good growing conditions, but more of their mass is allocated to protein.

However, individuals in relatively slow growing populations will be smaller on average than similarly aged conspecifics in fast growing populations. This continuum in growth strategies was apparent in comparisons between anadromous and barriered streams within regions and comparisons between regions.

Seasonal changes in growth and energy content

Juvenile fish in high latitude streams are believed to have distinct lipid phenologies characterized by significant increases in lipid content by the end of the summer and subsequent declines over winter. This pattern has been described for a number of species including Atlantic salmon, brown trout (Berg and Bremset 1998), striped bass (Hurst and Conover 2003), arctic char (Finstad et al. 2003) and Chinook salmon (Beckman et al. 2000). The data described here are somewhat consistent with that pattern. Energy content of Dolly Varden in both stream types peaked in August or October. In addition, there were significant declines in the energy content of Dolly Varden from the Homer streams over the winter of 2004-2005.

The growth patterns described here are also consistent with previous reports describing seasonal changes in the growth of fluvial salmonids (Quinn 2005). RNA/DNA ratios in both stream types indicated a decline in growth from peaks in spring and early summer to minima in mid to late summer. Similar to the RNA/DNA ratios, the growth rate of age 0+ Dolly Varden was greatest before August and subsequently decreased. Declining growth in mid to late summer is consistent with observations of increased stream temperatures and decreasing prey availability in the Homer (Mauger 2005) and Juneau (Lessard and Merrit 2006) streams. Increased temperatures would increase resting

metabolic rates and consequently consumption rates, but decreased food supply would limit the amount of energy available for growth.

Growth and condition in barriered and anadromous streams

Within regions, the presence of anadromous populations was associated with poor condition in resident Dolly Varden. Poor condition was typified by reduced growth, energy and lipid content. These reductions in condition added to the regional differences so that fish from the anadromous stream in Juneau were consistently in the poorest condition. While differences between stream types were not always significant, fish in the anadromous streams always had lower average size at age, growth, energy density, allocations of mass to lipid and length-adjusted energy contents than their counterparts in barriered streams (Table 4.6). Levels of these characters in fish from the anadromous stream in Homer were often equal to or higher than those of the barriered stream in Juneau. This suggests that underlying nutrient levels in steams can ameliorate the growth and condition limitations associated with the presence of anadromous populations.

Temperature differences between streams also do not account for differences in growth rates. The relatively poor growth of the fish in the Juneau stream occurred at average stream temperatures of less than 12°C. Takami et al. (1997) identified 19° C as the upper limit at which Dolly Varden cease feeding in response to temperature and Lyytikainen et al. (1997) reported 15° C as optimal for growing arctic char yearlings. Thus it is unlikely that poor growth in the anadromous stream near Juneau resulted from temperature stress. In addition, stream temperatures were lower in Homer streams and the barriered stream near Juneau, but fish in those streams had higher growth.

It is more likely that growth differences relate to the amount of energy available to the different populations. A feature common to both pairs of streams is the increased diversity of fish species in the anadromous streams relative to the barriered streams. This increased diversity included increased numbers of piscine predators that do not exist in the barriered streams. Recall that age 0 Dolly Varden averaged approximately 30 mm during summer, making them vulnerable to larger steelhead, chinook or coho salmon predation. In addition, these same species would have been competitors with older Dolly Varden. Consequently, foraging success in the anadromous streams was likely reduced relative to that in the barriered streams. While the density of salmonids may be constant for a given stream regardless of diversity, Dolly Varden in more diverse systems may experience decreased growth through increased interference competition (Taniguchi and Nakano 2000). Volk (2004) determined that streams bordered with nitrogen fixing alder, such as Sheep Creek, have greater algal and invertebrate biomass than streams shaded by a dense conifer overstory, such as Shrine Creek. However, surveys of anadromous and barriered streams near Juneau failed to detect differences among streams in insect density (Lessard and Merrit 2006). Note that reductions in growth resulting from reduced foraging success would exacerbate reductions related to differences in the inherent productivity of streams.

Effects of adult salmon on resident Dolly Varden

Fatty acid compositions indicated little evidence of direct movement of marine lipid into Dolly Varden tissues. While fatty acid compositions of Dolly Varden in both of the anadromous streams were more similar to marine archetypes than those in barriered

streams, only a few fish in each stream appeared to shift their fatty acid compositions after carcass arrival. Rapid and large shifts in fatty acid composition would be expected following carcass arrival if the trophic linkage between the fish and carcass tissues was relatively short (i.e. direct consumption) as a result of the differences in fatty acid composition between terrestrial and marine energy sources (Henderson and Tocher 1987). Direct consumption of the marine lipids and the Dolly Varden would allow much of the marine fatty acid composition to be conserved. Instead the less dramatic differences identified here indicate incorporation of marine lipids through a longer trophic chain (i.e. indirect consumption) in which much less of the composition is conserved. Indirect consumption implies benefits derived from marine-derived energy, if they exist, would be delayed in higher level consumers. This delay would serve to prolong the growing season by extending the period in which energy is available to top level consumers. In the anadromous stream near Juneau this may have been the case. Peak levels of dry mass and energy content in Dolly Varden from Shrine Creek were not evident until October, coincident with post-spawning peaks in epilithon dry mass (Cak 2005).

Examination of the rates at which growth changed during late summer suggests that the presence of adult salmon supported the growth of Dolly Varden in anadromous streams. Adult salmon could potentially increase growth potential by increasing prey availability during redd excavation (Minkawa 1997), reducing competition by providing an alternative food supply (Scheuerell et al. 2007), or by supplying nutrients to producers when they spawn and die (Chaloner et al. 2002). Adult salmon arrived in the Juneau

streams a few days following the July sample and a significant number of carcasses were observed during sampling in August. Growth on a wet mass basis of age 0+ fish collected from the anadromous stream near Juneau increased more than threefold between July and August compared with a doubling in the barriered stream. Moreover, growth in length did not differ between stream types after adults arrived in July. Sustained growing conditions in anadromous streams were also indicated by RNA/DNA ratios. Fish in the anadromous stream near Juneau increased RNA/DNA ratios from 1.8 to 2.4 between July and August, while values declined or remained constant in the barriered stream. Weber et al.(2003) reported RNA/DNA ratios of less than 1.5 for starving rainbow trout and ratios greater than 3.0 for actively growing fish. RNA/DNA ratios of fish from the anadromous stream near Homer increased between August and October, while those from the barriered stream remained constant.

The potential for carcasses to improve growing conditions for salmonids, including Dolly Varden has been previously demonstrated. Growth of Dolly Varden and cutthroat trout increased in reaches of Cedar Creek that were amended with carcasses (Wipfli et al. 2003). Similarly, addition of carcasses to Bridget Cove Creek resulted in increased growth of cutthroat trout (Wipfli et al. 2004). These studies established carcass effects by contrasting growth of fish in untreated reaches with that of fish in treated reaches in late summer and early fall. It is not known if the growth of fish in the various reaches was the same prior to carcass addition. However, in late summer and early fall the biomass and density of insect populations in these streams were likely declining (Lessard and Merrit 2006). Carcass additions could have improved forage conditions, but

not by dislodging benthic insects because no redds were excavated. Dolly Varden in the untreated reach of the Cedar Creek study increased their wet mass by 0.39 % between September and October compared to 1.90 % for fish in the amended reach (Wipfli et al. 2003). For comparison, age 0+ Dolly Varden in the anadromous stream near Juneau increased their mass by 0.55 % between August and October.

Further evidence for improved growth following adult immigration can be identified in the dry mass and proximate composition data for the Juneau streams. Length-adjusted dry mass declined for each of the populations studied here between August and October except for those in the anadromous stream near Juneau. Fish in this stream gained mass and increased RNA /DNA ratios by 30%. In contrast, fish in the barriered stream near Juneau lost mass and increased their RNA/DNA ratios slightly. The contrasting trajectories for mass between August and October caused differences in length-adjusted energy content between these groups observed in August to disappear by October. This change was due to increased mass rather than alterations in the composition of the mass as indicated the constancy of the mass specific energy content. During this same period adult salmon entered the stream, spawned and then died.

These effects are in contrast to those observed among Dolly Varden that directly incorporated marine-derived lipids into their tissues. Direct incorporation was indicated by substantial shifts in fatty acid composition to one more similar to that of the marine archetypes. Such saltatory changes in fatty acid composition as have been described for coho salmon (Heintz et al. 2004). These changes in Dolly Varden fatty acid composition were accompanied by increases in lipid and growth, consistent with reports of increased

lipid in growth in Dolly Varden (Wipfli et al. 2003) and cutthroat trout (Wipfli et al. 2004). Thus, direct consumption of marine-lipids appears to result in substantive changes in the growth and energy allocation strategies of juvenile salmonids.

In conclusion, the presence of carcasses was associated with diminished energy intake in resident Dolly Varden. This led to a growth strategy that favored increases in mass over length and allocations of mass to protein over lipid, characteristics of fish in poor nutritional condition. Fatty acid analysis indicated a marine influence on Dolly Varden found in anadromous streams, but these marine-derived lipids there were likely incorporated through long trophic linkage between the carcass tissues and the Dolly Varden. In contrast, some individuals appeared to benefit from a much shorter trophic linkage. These individuals had higher growth and improved lipid stores relative to conspecifics sampled at the same time. Ultimately those few individuals that incorporated marine-derived lipids through direct processed found themselves in improved nutritional condition.

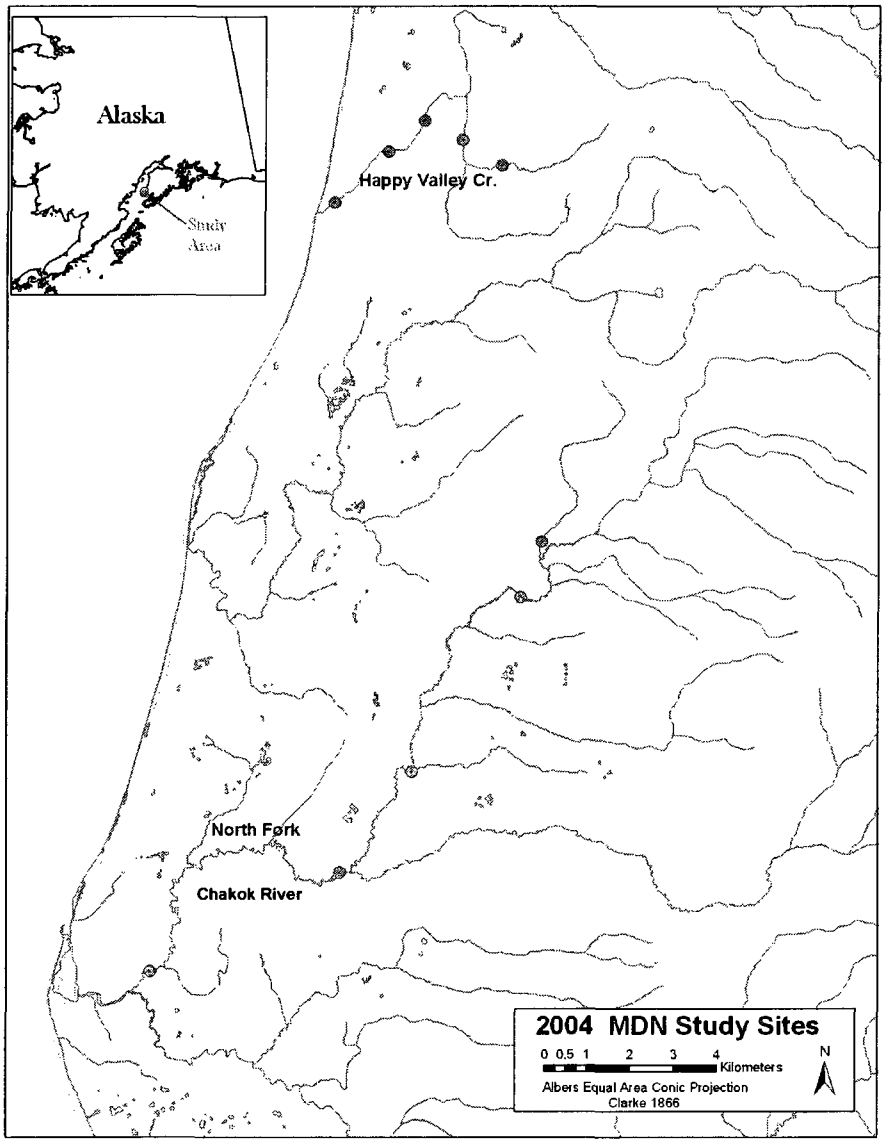

Figure 4.1. Location of streams sampled near Homer, AK. The Chakok River is the anadromous stream and Happy Valley Ck is the barriered stream. Markers show sampling stations.

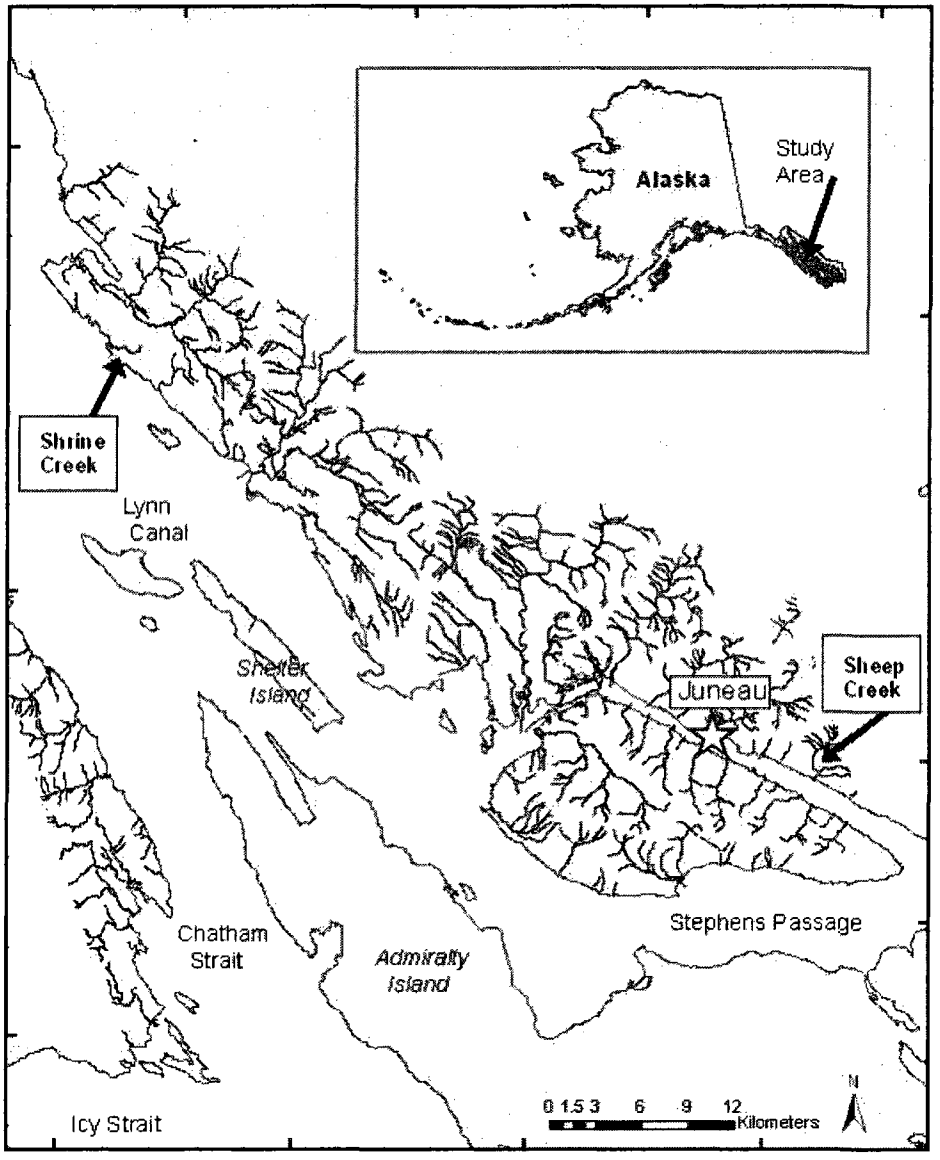

Figure 4.2. Location of streams near Juneau, AK. Shrine Creek is the anadromous stream and Sheep Creek is the barriered stream. Sampling stations not visible at the scale shown.

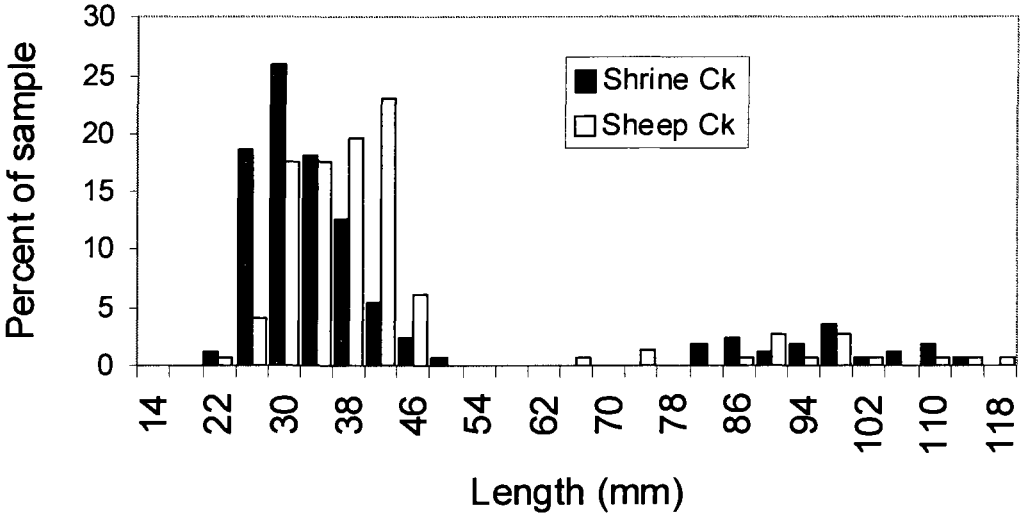

Figure 4.3. Length frequency distributions for fish collected near Juneau. Figure shows fish from the anadromous (filled symbols) and barriered (open symbols) streams during the summer of 2004.

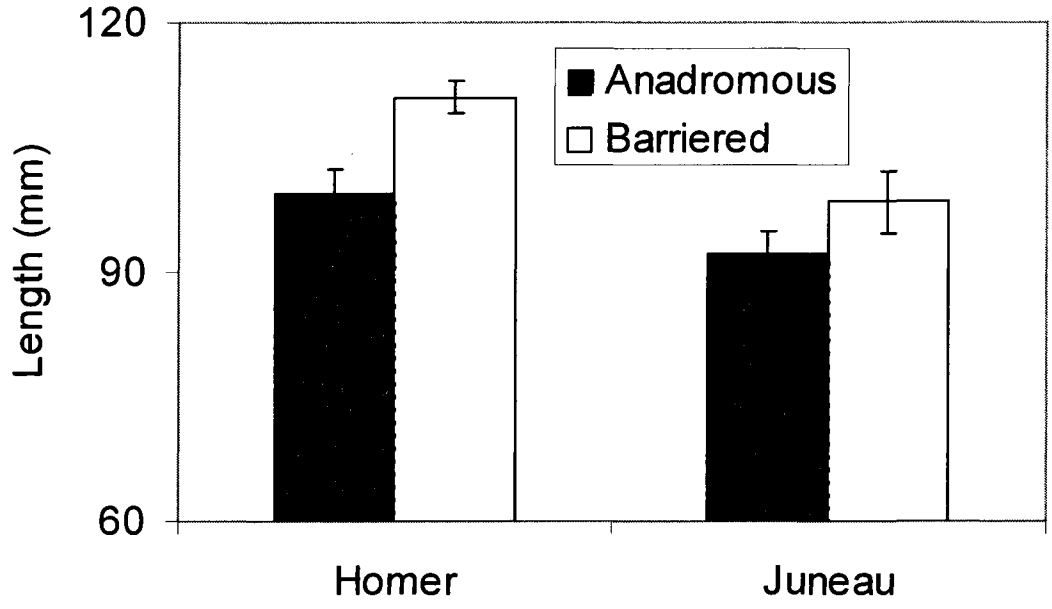

Figure 4.4. Size-at-age for age-2 Dolly Varden. Bars depict mean lengths (± 1 s.e) of fish from anadromous (filled symbols) and barriered streams (open symbols) located near Homer and Juneau, AK.

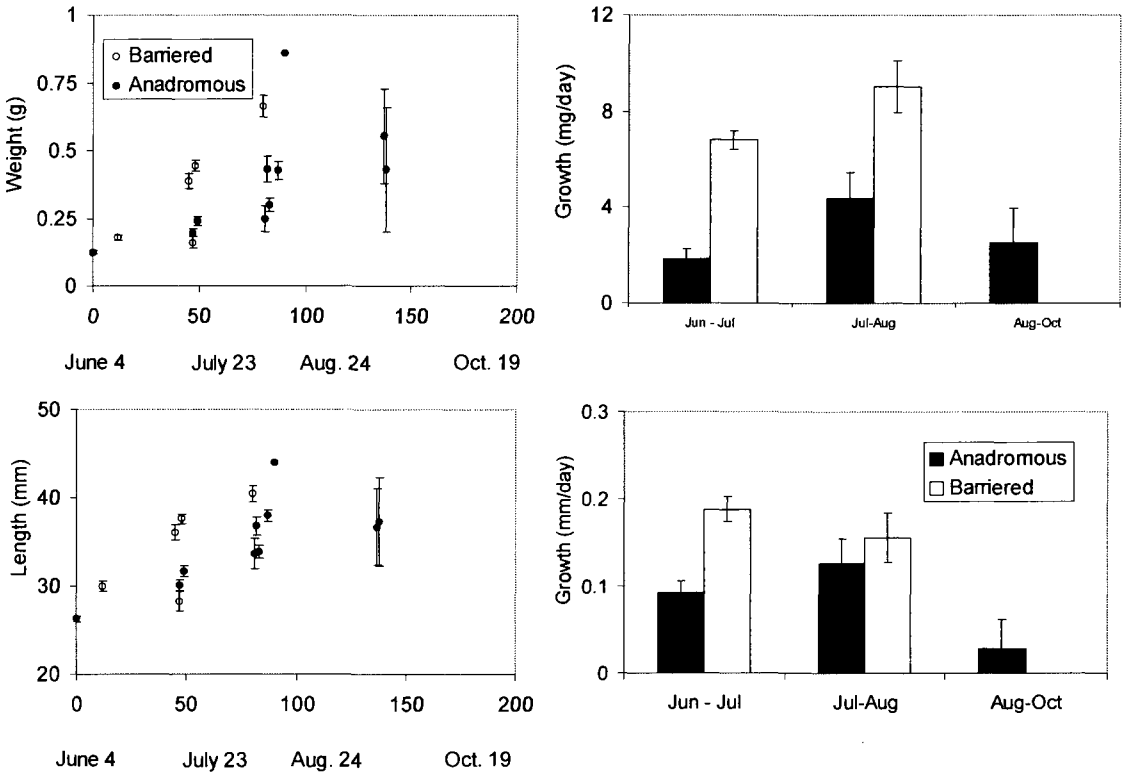

Figure 4.5. Size and growth of age-0 Dolly Varden. Panels show mean size (± 1 s.e.) (Left hand panels) and growth (Right hand panels) of age-0 Dolly Varden collected from anadromous (solid symbols) and barriered (open symbols) streams near Juneau, AK in 2004. Each point refers to the mean size of all fish collected on a given day. Note the single age-0 fish collected from the barriered steam in October is not shown.

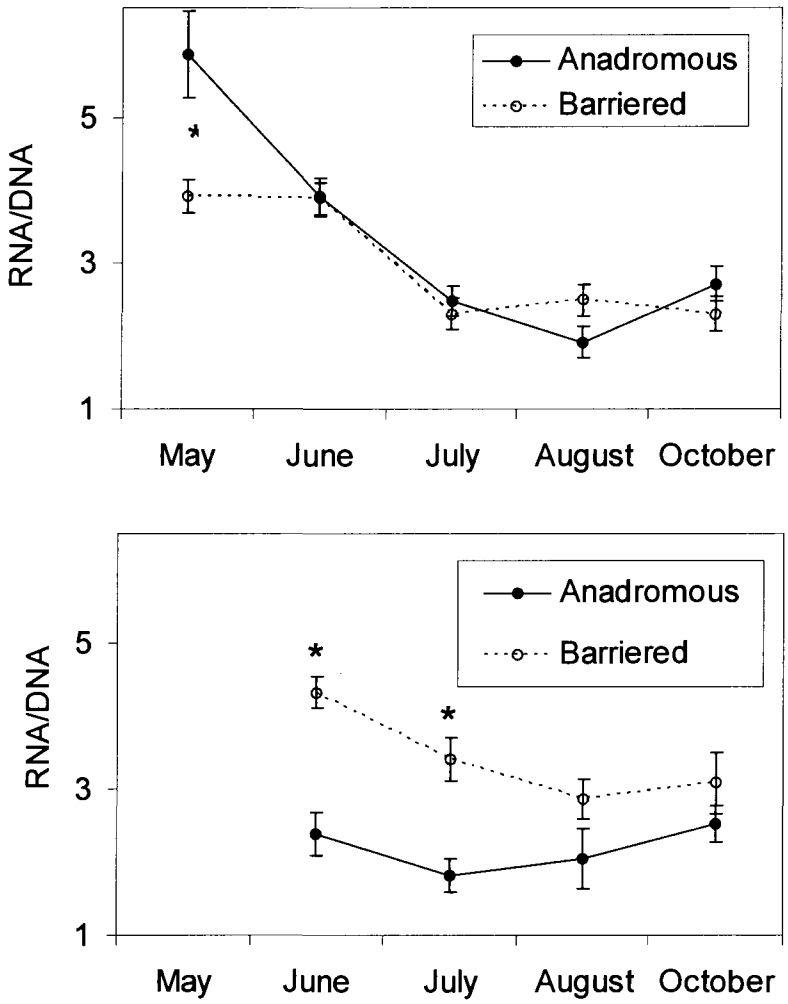

Figure 4.6. Seasonal changes in RNA/DNA ratios. Charts show mean RNA/DNA ratios (± 1 s.e.) of Dolly Varden from anadromous (filled symbols) and barriered (open symbols) streams near Homer (upper panel) and Juneau (lower panel) AK in summer 2004. Asterisks identify collections that differ significantly ($P < 0.05$).

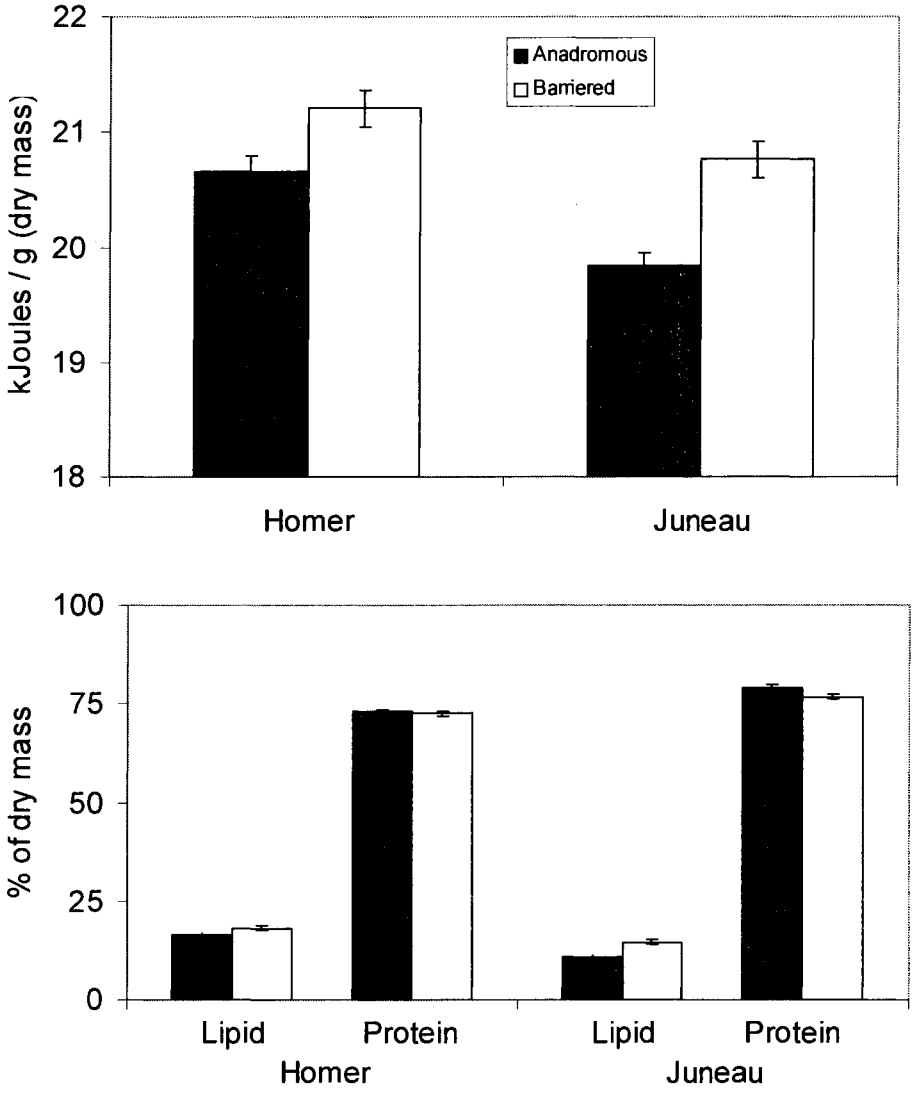

Figure 4.7. Body composition of Dolly Varden. Top Panel: Mass specific energy content (dry mass) of Dolly Varden from anadromous (filled symbols) and barriered streams (open symbols) from Homer and Juneau in summer 2004. Lower Panel: Allocations of dry mass to lipid and protein in the same Dolly Varden as the top panel.

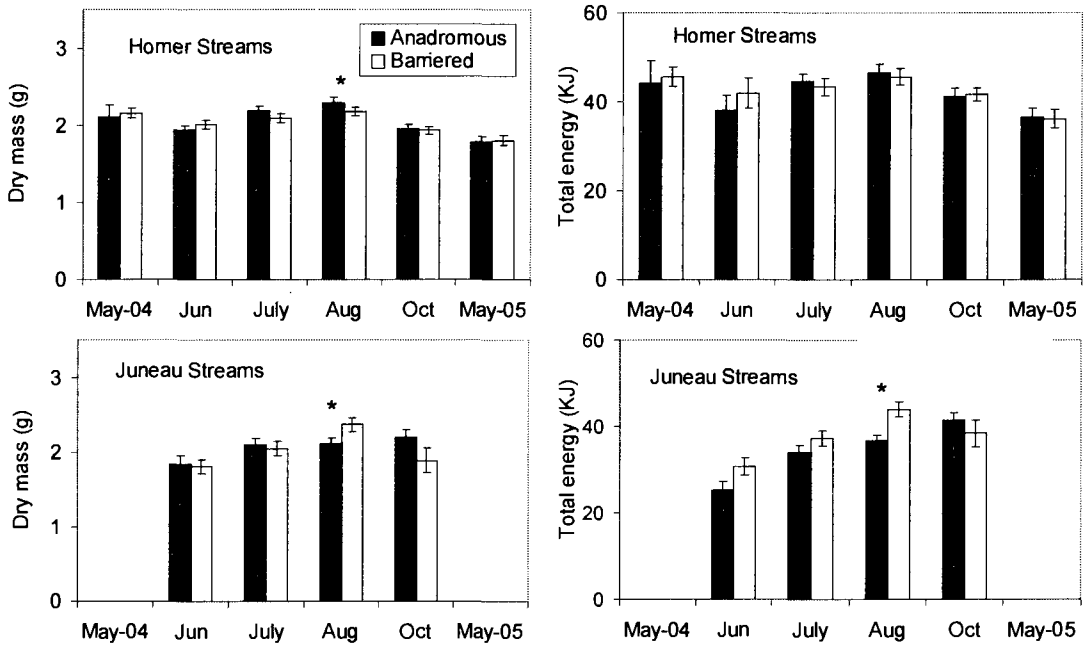

Figure 4.8. Dry mass and energy content of Dolly Varden in summer 2004. Left hand panels: Length adjusted dry mass of a 105 mm Dolly Varden from anadromous (filled symbols) and barriered streams (open symbols) collected near Homer (top) and Juneau (bottom) in summer 2004. Right hand panels: Length adjusted energy content of 105 mm Dolly Varden during summer 2004. Panels arranged as on lefthand side. Asterisks denote months in which barriered and anadromous streams differ ($P < 0.045$).

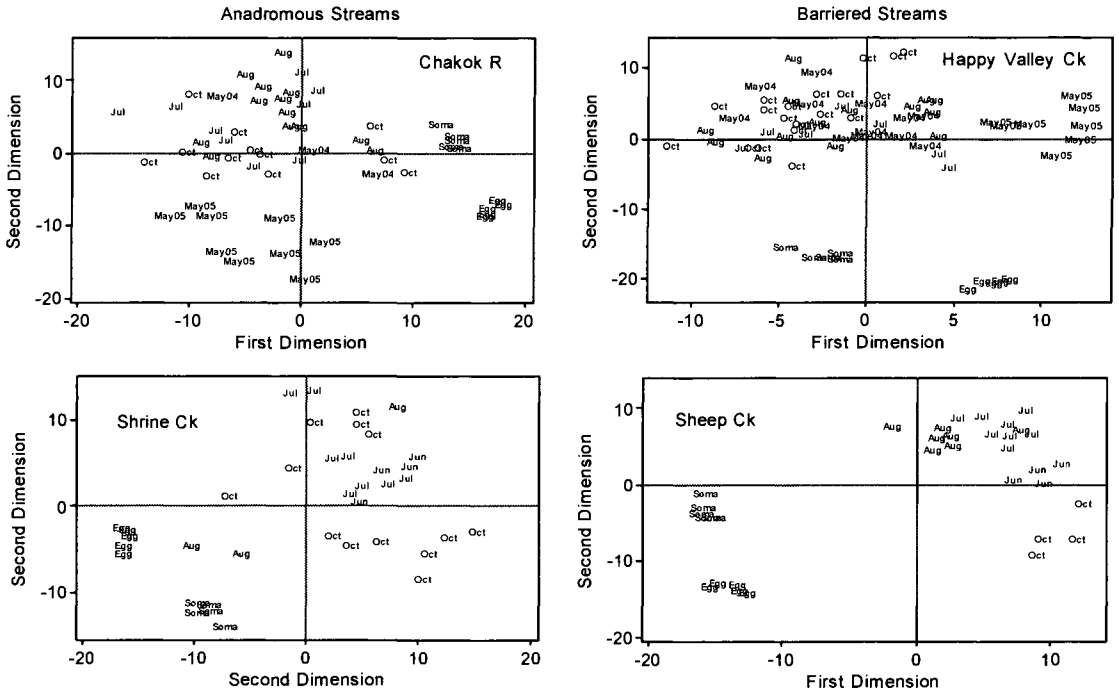

Figure 4.9. Seasonal changes in Dolly Varden fatty acids. Non-metric multidimensional scaling plots (Kruskall's stress < 0.187) show fish from anadromous (left column) and barriered (right column) streams. Two models are shown, one for streams near Homer (top row) and the other for streams near Juneau (bottom row). Individual streams are shown to aid in viewing sample positions. Symbols depict the month in which each fish was sampled during 2004, those labeled "Egg" and "Soma" depict the position of chinook salmon tissues taken from the Chakok River. Note Dolly Varden were sampled in May 2004 and 2005 from the streams near Homer.

Table 4.1. Reported characteristics of streams used in this study. Ranges for nutrients and discharge reflect extreme values reported for the months before and after the respective salmon runs. Temperatures reflect average between May and August. Abbreviations: Anad.: anadromous, Ch: Chinook salmon, Pk: Pink salmon, Cm: Chum salmon, Co: Coho salmon.

		Homer Streams		Juneau Streams	
Characters		Chakok R	Happy V Ck	Shrine Ck	Sheep Ck
Type		Anad.	Barriered	Anad.	Barriered
Drainage area	(Km^2)	38.1	7.74[a]	Unknown	4.57[a]
Discharge range	(CFS)	178-59[b]	8.5-7.75[b]	1.17	71-83[a]
Avg. temp °C	(Jun –Oct)	8.9	Unknown	9.67	7.35
NH_4^+	(µg/L)	12.9[c]- 13.3	9.4[b]- 21.9	4[d]- 31	5[e]-18
Dissolved PO_4	(µg/L)		23.7[b]- 33.8	2[d]- 63	2[e]- 2
Adult salmon species and timing		Ch: Jul-Aug Pk: Aug Co: Oct		Cm : Aug Pk : Sep Co: Oct	

a. USGS (2006)

b. Rinella (personal communication)

c. Mauger (2005)

d. Mitchell and Lamberti (2005)

e. (USGS 2006)

f. Cak (2005)

Table 4.2. Size and age of Dolly Varden used in this study. Shown are the number, mean (± 1 s.e.) size and range of ages of fish collected from anadromous and barriered streams between May 2004 and 2005.

	Sample Period	Dates Sampled	N	Average Length (mm)	Average Weight (g)	Age (Years)
Homer Streams						
Anadromous (Chakok River)	May '04	May 21-25	3	101±4.0	9.6±1.6	3 – 5
	June '04	Jun 17-18	10	92± 4.9	7.2±1.3	1 – 4
	July '04	Jul 20-21	16	102±5.2	11.4±1.3	1 – 3
	Aug '04	Aug 24-26	15	107±5.2	13.5±1.6	1 – 4
	Oct. '04	Oct 20-27	12	109±4.7	11.8±1.6	0 – 4
	May '05	May 12-28	9	96±7.9	8.7± 2.3	Not measured
Barriered (Happy Valley Ck)	May '04	May 27-29	13	118± 8.1	17.9±3.5	2 – 4
	June '04	Jun 18	14	100±2.2	9.2±0.6	1 – 3
	July '04	Jul 20-21	15	118±4.0	16.6±1.9	2 – 4
	Aug '04	Aug 18-20	15	107±3.8	11.6±1.2	1 – 3
	Oct. '04	Oct 20-22	19	104±7.6	13.2±2.8	0 – 3
	May '05	May 17-25	8	108±10.7	13.5±4.0	Not measured

Table 4.2, continued.

	Sample Period	Dates Sampled	N	Average Length (mm)	Average Weight (g)	Age (Years)
Juneau Streams						
Anadromous (Shrine Ck)	June '04	Jun 4-22	9	55.2±10.9	3.2 ± 01.2	0 – 3
	July '04	Jul 19-29	18	54.7±7.3	3.3 ± 01.1	0 – 4
	Aug '04	Aug 24-Sep 2	24	57.0 ± 6.0	3.2 ± 00.9	0 – 4
	Oct. '04	Oct 19-22	14	72.5 ± 6.9	4.7 ± 01.1	0 – 5
Barriered (Sheep Ck)	June '04	Jun 16-24	10	55.7 ±10.8	3.2 ± 1.4	0 – 1
	July '04	Jul 14	14	45.8 ±5.1	1.3 ± 0.6	0 – 2
	Aug '04	Aug 23	18	60.6 ±6.0	3.4 ± 0.9	0 – 2
	Oct. '04	Oct 25	4	95.5 ±11.8	8.3 ± 2.5	0 – 1

Table 4.3. Size and growth of age-0 Dolly Varden. Shown are mean (± 1 s.e.) forklengths, weights and estimated growth rates of age-0 Dolly Varden collected in Shrine and Sheep Creeks near Juneau in 2004. Values in bold denote different ($P < 0.04$) growth in the two streams during the corresponding period.

Stream	Median Collection Date	n	Forklength (mm)	Weight (g)	Growth (mm x day^{-1})	Growth (mg x day^{-1})
Shrine Ck	June 4	55	26.3 ± 0.3	0.125 ± 0.005		
(Anadromous)	July 22	34	31.0 ± 0.5	0.222 ± 0.011	**0.09**	**1.9**
	August 28	46	35.6 ± 0.6	0.369 ± 0.028	0.13	**4.4**
	Oct. 19	6	38.2 ± 2.7	0.572 ± 0.100		
Sheep Ck	June 16	36	30.0 ± 0.5	0.179 ± 0.011		
(Barriered)	July 20	77	36.0 ± 0.5	0.392 ± 0.018	**0.18**	**6.7**
	August 23	18	40.4 ± 0.9	0.663 ± 0.039	0.15	**9.0**
	Oct. 25	1	66.0	2.311		

Table 4.4. Differences in length-adjusted dry mass of Dolly Varden. Table shows results of pairwise comparisons of fish from anadromous and barriered streams in each of the months sampled. Values in bold represent significant differences ($P < 0.05$).

	% Difference in Means $\left(\frac{Anad. - Barriered}{Anad.}\right) \times 100$	N	MSE	F
Homer Streams				
May '04	-2.5	2	0.0019	0.0614
June	-9.4	10	0.0019	0.6992
July	4.2	15	0.0019	1.4250
August	5.1	15	0.0019	2.0340
October	0.6	12	0.0019	0.0213
May '05	-0.9	8	0.0019	0.0288
Juneau Streams				
June	2.3	9	0.005	0.092
July	2.5	14	0.005	0.163
August	**-12.3**	18	0.005	**4.554**
October	14.1	4	0.005	1.758

Table 4.5. Differences in length-adjusted energy content of Dolly Varden. Table shows results of pairwise comparisons of fish from anadromous and barriered streams in each of the months sampled. Significant differences are shown in bold ($P < 0.01$).

	% Difference in Means $\left(\frac{Anad. - Barriered}{Anad.}\right) \times 100$	N	MSE	F
Homer Streams				
May '04	-3.3	2	0.005	0.044
June	-10.4	3	0.005	0.616
July	2.5	15	0.005	0.202
August	1.82	14	0.005	0.100
October	-1.2	12	0.005	0.033
May '05	0.9	8	0.005	0.014
Juneau Streams				
June	-21.9	5	0.005	4.109
July	-9.4	13	0.005	2.197
August	**-19.9**	**18**	**0.005**	**12.482**
October	7.3	4	0.005	0.484

Table 4.6. Summary of ANOVAs comparing streams. Asterisks denote range of P values: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Response	Homer	Juneau
Length-at-Age	Anad. < Bar'd**	Anad. < Bar'd
Age-0 growth	Not measured	Anad. < Bar'd*
Mass specific energy	Anad > Bar'd	Anad < Bar'd***
%lipid	Anad. < Bar'd	Anad. < Bar'd***
% protein	Anad. > Bar'd	Anad. > Bar'd*
Length-adjusted dry mass	Anad. < Bar'd	Anad. < Bar'd
Length-adjusted energy	Anad. < Bar'd	Anad. < Bar'd*

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Appendices

Appendix 4.1. Fatty acid composition of Dolly Varden in July. Values are relative concentrations. Fatty acids marked with "*" were used to calculate dissimilarity indices (equation 4.5). n.d.= not detected; n.e. = not examined.

		Happy Valley		
Fatty Acid[a]	Chakok R	Ck	Shrine Ck	Sheep Ck
12:0	0.44 ± 0.34	0.30 ± 0.18	0.22 ± 0.17	0.27 ± 0.11
14:0*	2.78 ± 2.00	2.50 ± 0.58	1.02 ± 0.31	2.66 ± 0.54
14:1ω5	0.48 ± 0.20	0.50 ± 0.19	0.05 ± 0.05	0.06 ± 0.05
15:0*	0.20 ± 0.12	0.29 ± 0.08	0.35 ± 0.08	0.21 ± 0.05
16:0*	15.23 ± 1.32	14.48 ± 1.22	10.31 ± 2.06	11.30 ± 0.86
16:1ω7*	16.45 ± 2.63	11.87 ± 2.79	4.34 ± 1.50	4.98 ± 0.62
17:0*	0.46 ± 0.15	0.95 ± 0.35	1.14 ± 0.43	0.29 ± 0.10
17:1ω7	0.28 ± 0.16	0.51 ± 0.21	0.49 ± 0.11	0.28 ± 0.07
18:0*	4.21 ± 0.53	4.67 ± 0.25	5.80 ± 0.55	4.38 ± 0.09
18:1ω11*	1.13 ± 2.42	0.23 ± 0.08	0.15 ± 0.07	0.03 ± 0.01
18:1ω9c*	21.30 ± 5.20	25.00 ± 5.23	19.97 ± 4.18	17.00 ± 1.12
18:1ω7*	5.47 ± 1.82	7.44 ± 1.20	6.06 ± 1.11	5.10 ± 0.50
18:2ω6c*	7.21 ± 2.55	7.06 ± 2.86	6.53 ± 1.86	8.09 ± 1.31
18:3ω6*	0.42 ± 0.19	0.39 ± 0.08	0.27 ± 0.10	0.46 ± 0.09
20:0*	0.38 ± 0.20	0.25 ± 0.09	0.23 ± 0.08	0.68 ± 0.08

Appendix 4.1, continued

		Happy Valley		
Fatty Acid[a]	Chakok R	Ck	Shrine Ck	Sheep Ck
18:3ω3*	6.95 ± 1.90	6.53 ± 3.46	2.69 ± 0.89	7.19 ± 0.73
20:1ω11	0.74 ± 1.26	0.86 ± 0.56	1.39 ± 0.27	n.e.
20:1ω9	0.46 ± 0.24	0.68 ± 0.35	0.67 ± 0.09	n.e.
18:4ω3	2.93 ± 2.21	1.91 ± 1.12	0.51 ± 0.21	6.44 ± 2.04
20:2ω6*	0.49 ± 0.36	0.74 ± 0.30	0.91 ± 0.41	0.35 ± 0.11
20:3ω6*	0.53 ± 0.22	0.68 ± 0.25	1.14 ± 0.37	0.57 ± 0.15
22:0	0.45 ± 0.52	0.18 ± 0.09	0.23 ± 0.16	0.25 ± 0.02
20:3ω3	n.e.	n.e.	0.20 ± 0.14	n.d..
20:4ω6*	0.85 ± 0.41	1.50 ± 0.46	5.13 ± 0.94	1.51 ± 0.18
22:1ω11	0.00 ± 0.00	0.07 ± 0.19	0.35 ± 0.04	n.e.
22:1ω9	0.04 ± 0.08	0.03 ± 0.07	0.17 ± 0.03	n.e.
20:5ω3*	5.36 ± 3.12	5.65 ± 1.36	7.05 ± 1.74	9.94 ± 0.79
22:2ω6	0.01 ± 0.03	0.06 ± 0.08	0.09 ± 0.01	0.07 ± 0.01
24:0	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.06	0.00 ± 0.00
22:4ω6	0.00 ± 0.00	0.24 ± 0.31	1.18 ± 0.30	0.38 ± 0.06
24:1ω9	0.02 ± 0.05	0.00 ± 0.00	0.78 ± 0.39	0.40 ± 0.14
22:5ω3*	1.47 ± 0.95	1.07 ± 0.21	2.19 ± 0.40	1.85 ± 0.28
22:6ω3*	3.21 ± 2.02	3.27 ± 0.93	18.84 ± 8.06	15.27 ± 1.78

Appendix 4.1, continued.

		Happy Valley		
Fatty Acid[a]	Chakok R	Ck	Shrine Ck	Sheep Ck
Sum Sat'd	24.14 ± 1.85	23.63 ± 1.10	19.38 ± 2.13	20.04 ± 0.83
Sum Mono	45.19 ± 5.78	46.93 ± 7.27	33.50 ± 7.17	27.82 ± 1.46
Sum n3				
PUFA	19.92 ± 6.47	18.44 ± 6.41	31.28 ± 9.17	40.69 ± 2.79
Sum n6				
PUFA	9.50 ± 3.17	10.62 ± 2.56	15.15 ± 3.22	11.35 ± 1.73
ω3/ω6	2.70 ± 2.08	1.79 ± 0.76	2.30 ± 1.34	3.69 ± 0.83
N	10	7	8	8

a. Fatty acids are named by N:aωb where N refers to the number of carbons, a the number of double bonds and b the location of the first double bond from the methyl end.

Appendix 4.2. Fatty acid compositions of Dolly Varden in October. Values are relative concentrations. Fatty acids marked with "*" were used to calculate dissimilarity indices (equation 4.5). n.d.= not detected; n.e. = not examined.

	Happy			
Fatty Acid	Chakok R	Valley Ck	Shrine Ck	Sheep Ck
12:0	0.39 ± 0.22	0.39 ± 0.26	0.24 ± 0.16	0.43 ± 0.12
14:0*	2.71 ± 0.63	2.84 ± 0.86	1.33 ± 0.56	3.24 ± 1.25
14:1ω5	0.26 ± 0.15	0.47 ± 0.33	0.06 ± 0.07	0.32 ± 0.05
15:0*	0.40 ± 0.26	0.41 ± 0.20	0.34 ± 0.06	0.40 ± 0.14
16:0*	12.64 ± 1.16	14.68 ± 1.80	10.24 ± 1.15	13.24 ± 1.02
16:1ω7*	12.05 ± 3.03	11.12 ± 2.68	5.33 ± 2.49	7.08 ± 3.07
17:0*	0.63 ± 0.35	0.78 ± 0.22	1.55 ± 0.56	1.00 ± 0.35
17:1ω7	0.83 ± 0.48	0.76 ± 0.26	0.63 ± 0.26	0.17 ± 0.29
18:0*	4.29 ± 0.74	4.59 ± 0.57	6.18 ± 1.21	5.17 ± 1.27
18:1ω11*	0.44 ± 0.42	0.29 ± 0.27	0.33 ± 0.24	0.09 ± 0.06
18:1ω9c*	21.29 ± 4.38	21.34 ± 4.57	20.54 ± 5.60	24.47 ± 1.62
18:1ω7*	5.77 ± 1.16	5.91 ± 1.36	8.25 ± 2.47	5.89 ± 0.77
18:2ω6c*	6.85 ± 3.08	8.78 ± 2.34	6.00 ± 1.81	10.26 ± 3.17
18:3ω6*	0.54 ± 0.22	0.52 ± 0.16	0.31 ± 0.14	0.57 ± 0.09
20:0*	0.44 ± 0.25	0.39 ± 0.17	0.13 ± 0.05	0.23 ± 0.06
18:3ω3*	6.83 ± 3.51	9.35 ± 2.20	2.31 ± 0.96	8.62 ± 0.99

Appendix 4.2, continued.

		Happy		
Fatty Acid	Chakok R	Valley Ck	Shrine Ck	Sheep Ck
20:1ω11	0.36 ± 0.18	0.43 ± 0.29	1.62 ± 1.01	0.78 ± 0.19
20:1ω9	0.64 ± 0.23	0.41 ± 0.20	0.77 ± 0.51	0.49 ± 0.10
18:4ω3	2.86 ± 1.13	2.59 ± 0.91	0.89 ± 1.66	3.03 ± 0.92
20:2ω6*	0.57 ± 0.24	0.67 ± 0.27	1.13 ± 0.46	0.92 ± 0.14
20:3ω6*	0.61 ± 0.31	0.73 ± 0.29	1.34 ± 0.52	0.80 ± 0.11
22:0	0.40 ± 0.40	0.28 ± 0.29	0.07 ± 0.07	0.31 ± 0.11
20:3ω3	0.47 ± 0.24	0.61 ± 0.22	0.16 ± 0.06	0.52 ± 0.06
20:4ω6*	1.32 ± 0.41	1.55 ± 0.32	5.03 ± 2.59	2.03 ± 0.37
22:1ω11	0.19 ± 0.27	0.05 ± 0.07	0.61 ± 0.72	0.29 ± 0.15
22:1ω9	0.25 ± 0.30	0.08 ± 0.15	0.19 ± 0.15	0.26 ± 0.09
20:5ω3*	6.08 ± 1.85	4.87 ± 1.89	6.59 ± 2.63	4.76 ± 2.22
22:2ω6	0.13 ± 0.22	0.06 ± 0.08	0.17 ± 0.21	0.22 ± 0.15
24:0	0.51 ± 0.62	0.20 ± 0.40	0.05 ± 0.10	0.29 ± 0.21
22:4ω6	0.27 ± 0.24	0.23 ± 0.16	1.24 ± 0.84	0.20 ± 0.23
24:1ω9	0.26 ± 0.31	0.20 ± 0.35	0.44 ± 0.44	0.34 ± 0.14
22:5ω3*	2.18 ± 1.12	1.47 ± 0.66	2.09 ± 1.06	1.17 ± 0.26
22:6ω3*	6.52 ± 4.57	2.95 ± 0.70	13.62 ± 9.98	2.41 ± 0.61

Appendix 4.2, continued.

Fatty Acid	Chakok R	Happy Valley Ck	Shrine Ck	Sheep Ck
Sum Sat'd	22.42 ± 1.91	24.55 ± 2.56	20.14 ± 2.34	24.30 ± 1.27
Sum Mono	41.65 ± 4.51	40.69 ± 3.80	38.25 ± 10.39	39.84 ± 1.35
Sum n3 PUFA	24.48 ± 6.81	21.23 ± 4.07	25.50 ± 12.51	19.99 ± 3.86
Sum n6 PUFA	10.15 ± 3.99	12.48 ± 3.07	15.06 ± 4.33	14.78 ± 3.46
ω3/ω6	3.33 ± 2.75	1.91 ± 0.93	1.95 ± 1.56	1.48 ± 0.73
N	12	19	13	4

Appendix 4.3. Fatty acid composition of Chinook salmon. Values are relative concentrations of total fatty acids in adult salmon returning to anadromous streams. Fatty acids marked with "*" were used to calculate dissimilarity indices (equation 4.5). n.d.= not detected; n.e. = not examined.

Fatty Acid	Chinook Soma	Chinook eggs
12:0	0.02 ± 0.01	0.02 ± 0.01
14:0*	3.31 ± 0.33	2.43 ± 0.33
14:1ω5	0.08 ± 0.01	0.07 ± 0.01
15:0*	0.31 ± 0.05	0.29 ± 0.05
16:0*	10.44 ± 0.61	8.07 ± 0.61
16:1ω7*	5.62 ± 0.73	6.41 ± 0.73
17:0*	0.34 ± 0.03	0.20 ± 0.03
17:1ω7	0.38 ± 0.08	0.47 ± 0.08
18:0*	3.80 ± 0.16	3.68 ± 0.16
18:1ω11*	1.13 ± 0.17	1.34 ± 0.17
18:1ω9c*	35.30 ± 3.48	31.50 ± 3.48
18:1ω7*	3.34 ± 0.21	3.78 ± 0.21
18:2ω6c*	1.37 ± 0.11	1.08 ± 0.11
18:3ω6*	0.06 ± 0.01	0.06 ± 0.01
20:0*	0.11 ± 0.01	0.02 ± 0.01
18:3ω3*	0.81 ± 0.08	0.77 ± 0.08

Appendix 4.3, continued.

Fatty Acid	Chinook Soma	Chinook eggs
20:1ω11	5.27 ± 1.63	0.51 ± 1.63
20:1ω9	4.20 ± 0.35	1.19 ± 0.35
18:4ω3	0.99 ± 0.10	0.71 ± 0.10
20:2ω6*	0.23 ± 0.05	0.18 ± 0.05
20:3ω6*	0.11 ± 0.01	0.18 ± 0.01
22:0	0.04 ± 0.00	n.d.
20:3ω3	0.12 ± 0.12	0.12 ± 0.12
20:4ω6*	0.40 ± 0.05	1.06 ± 0.05
22:1ω11	6.22 ± 1.52	0.00 ± 1.52
22:1ω9	1.08 ± 0.11	0.07 ± 0.11
20:5ω3*	4.27 ± 0.59	11.86 ± 0.59
22:2ω6	0.02 ± 0.00	n.d.
24:0	0.04 ± 0.00	n.d.
22:4ω6	0.15 ± 0.01	0.28 ± 0.01
24:1ω9	1.01 ± 0.19	0.24 ± 0.19
22:5ω3*	1.83 ± 0.39	5.25 ± 0.39
22:6ω3*	7.58 ± 1.22	18.14 ± 1.22

Appendix 4.3, continued.

Fatty Acid	Chinook Soma	Chinook eggs
Sum Sat'd	18.42 ± 0.83	14.72 ± 0.66
Sum Mono	63.63 ± 1.59	44.40 ± 1.80
Sum n3 PUFA	15.59 ± 2.06	36.85 ± 1.64
Sum n6 PUFA	2.36 ± 0.13	2.85 ± 0.11
ω3/ω6	6.60 ± 0.90	12.94 ± 0.75
N	5	5

Chapter V

General Conclusions

Introduction

Adult salmon deliver energy to their natal streams in the form of carcass tissues and eggs and this energy is obtained and used by juvenile salmonids in a variety of ways. Chapter II introduced the concept of direct incorporation versus indirect incorporation. Direct consumption refers to the incorporation of marine fatty acids either by direct consumption or through a short trophic linkage between consumer and adult salmon tissue. A hallmark of direct consumption was a dramatic shift in the fatty acid composition of consumers coincident with the arrival of adult salmon. Indirect consumption refers to the incorporation of marine fatty acids through long and diffuse trophic chains. Direct consumption was indicated by increased similarity in fatty acid composition between Dolly Varden and salmon carcass tissues among fish collected from streams with adult salmon runs relative to those in streams with no salmon runs. In addition, there was no seasonal shift in fatty acid composition among fish in the streams with adult salmon runs indicating that the increased similarity resulted from the permeation of marine lipids throughout the local food webs. The benefits of marine-derived lipids on juvenile salmonids depends on the temperatures at which juveniles feed and on the route by which they incorporate the lipid. These are discussed below.

Effects of direct consumption

Coho salmon, chironomids and nemourids studied in Chapter II were universal in their acquisition of marine-derived lipid as demonstrated by quantitative shifts in their fatty acid compositions. A few of the Dolly Varden studied in Chapter IV also displayed similar shifts. Direct acquisition of marine-lipids was accompanied by increased allocations of mass in the form of lipid in both these groups, indicating a shift in energy allocation strategy. Lipid class analysis of coho salmon and chironomids in Chapter II indicated that this increased allocation of mass was the result of increased allocations of lipid to triacylglycerols. Hence, the incorporation of marine-derived lipids resulted in increased energy stores. These shifts in energy allocation coincided with increased growth as demonstrated by the change in size of coho salmon and chironomids from the mesocosms and increased average RNA/DNA ratios in the Dolly Varden. Growth likely resulted from increased food supplies, quality or the combination.

Diet effects on fasting

Direct consumption of carcass tissues led fish to store greater amounts of energy in the form of lipid and maintain relatively high metabolic rates during winter. In Chapter III fish that directly consumed carcass tissues drew disproportionately on their lipid reserves in order to meet the energetic costs of winter. In addition, fish with higher nutritional status as indicated by increased lipid reserves had higher metabolic rates than fish in relatively poor nutritional condition. Despite the higher metabolic rates, individuals that began winter with high lipid reserves exited a fast with more muscle mass intact. These data indicate that down-regulating metabolic rate is likely the strategy

of last resort for overwintering salmonids. The focus on increased allocations of mass to protein observed among Dolly Varden in Chapter IV suggests that these individuals are likely forced to down-regulate metabolic rates over winter. Down-regulating metabolic rate over winter may have implications for survival due to increased disease susceptibility.

Effects of indirect consumption

Most of the Dolly Varden from the anadromous streams studied in Chapter IV acquired marine-derived lipids indirectly and they accrued few obvious benefits. Over most of the summer their growth and energy content was depressed relative to conspecifics in nearby barriered streams. Only after the arrival of carcasses did the dry mass or length-adjusted energy content of Dolly Varden in the anadromous streams equal or exceed those in the barriered streams. This potential effect of carcasses was an indirect benefit of a prolonged growing period. Presumably, marine-derived energy prolonged the production cycle in streams with adult salmon runs, allowing fish to continue growing. However, for most of the growing season Dolly Varden in streams with adult salmon runs experienced depressed growth. The relatively poor growth among Dolly Varden in the streams with adult salmon runs was accompanied by a growth strategy in which fish maximized mass gain over increases in length and protein gain over lipid. The prolonged growth period in streams with adult salmon runs enabled Dolly Varden of a given length to end the summer with energy contents higher than or equal to similarly sized conspecifics in barriered streams. However, this amounted to making the "best of a bad lot" in that size-at-age was larger in streams without adult salmon runs.

Carcasses support anadromy in juvenile salmonids

The presence of adult salmon carcasses in fluvial habitats reinforces the anadromous behavior of juvenile salmonids existing on high nutritional planes. Jobling (1994) identified "nutritional planes" as different feeding levels. He noted that the nutritional planes relate directly to metabolic rates during fasting. The data provided in Chapter III refines that idea by illustrating that the nutritional value of food also contributes to metabolic rate during fasting. The resident Dolly Varden in the anadromous streams studied in Chapter IV likely occupied low nutritional planes. These fish grew slowly and allocated relatively little of their dry mass to lipid. In contrast, the wild coho salmon described in Chapter II occupied a high nutritional plane as indicated by their relatively large allocations of mass to lipid. Results from Chapter III indicate that these differences in nutritional plane are likely to be accompanied by divergent metabolic rates. This divergence suggests resident salmonids are associated with low metabolic rate while the reverse is true for anadromous salmonids. In some species, such as coho salmon, resident forms are exceedingly rare while in other species, such as Dolly Varden, resident forms are commonly encountered. These observations are consistent with the observation that resident brook trout had low metabolic rates and occupied low energy habitats relative to anadromous forms in the same streams (Morinville 2005). In that report low energy habitats were considered those with reduced stream flow and hence availability of drift. Food was more available in the high energy habitats, but activity costs were also higher due the need to maintain position in higher currents. By down-regulating metabolic rate, resident forms could complete their life history in the stream.

In contrast, increased growth in the high energy habitat meant that total metabolic demand outstripped the availability of energy availability forcing fish to emigrate seaward in search of more food.

Fish in anadromous streams can adopt a variety of life history strategies based on their ability to maintain position in high energy habitats. Fish on high nutritional planes can maintain higher metabolic rates, facilitating foraging and sparing of muscle mass during winter. Salmon carcass tissues and eggs offer a high value energy source to those individuals through direct consumption. In contrast, carcasses offer little direct benefit to those individuals occupying low nutritional planes in suboptimal habitats. Carcass tissues have minimal value to these individuals except for their contributions to bottom-up processes, competitive release or reduced predation risk. Over winter, these individuals are likely to reduce foraging and draw on muscle mass to meet metabolic demand. The data provided here indicate that the direct benefits result from the relatively high lipid to protein ratio offered by eggs and carcass tissues. This suggests improved lipid to protein ratios in prey fauna could therefore be an alternative benefit of marine energy.

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